The Distracted Brain

The neurobiology and neuropsychology of attention-deficit/hyperactivity problems in the general population

Sabine Mous

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ISBN: 978-94-6259-743-3

Cover design and layout: LenShape (Len 't Hoen), Den Haag, the Netherlands
Printing: Ipskamp drukkers, Enschede, the Netherlands

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Het verwarde brein

De neurobiologie en neuropsychologie van aandachts- en hyperactiviteitsproblemen in de algemene populatie

Proefschrift

ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam
op gezag van de
rector magnificus

Prof.dr. H.A.P. Pols

en volgens besluit van het College voor Promoties.
De openbare verdediging zal plaatsvinden op

dinsdag 1 september 2015 om 15.30 uur

door

Sabine Elise Mous
geboren te Schiedam

Erasmus University Rotterdam

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CHAPTER

1 General introduction

Attention-deficit/hyperactivity disorder

Attention-deficit/hyperactivity disorder (ADHD) is one of the most common neurodevelopmental disorders, with a worldwide prevalence of 3-5% (Polanczyk, Willcutt, Salum, Kieling, & Rohde, 2014). ADHD is characterized by a persistent pattern of age-inappropriate attention problems and symptoms of hyperactivity and/or impulsivity (American Psychiatric Association, 2013).

The existence of a continuum in ADHD

The notion that child psychopathology, such as ADHD, might be better described within a dimensional framework, rather than with clearly defined diagnostic categories, has recently gained support. Within this framework of continuous symptom levels, children with clinical disorders constitute the extreme end of the spectrum, as depicted in Figure 1.1.

FIGURE 1.1. The dimensionality of child psychopathology

In line with this, the dimensionality of (developmental) psychiatric disorders is now considered in the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) (American Psychiatric Association, 2013) and dimensional approaches are evaluated as a part of the National Institutes of Mental Health's Research Domain Criteria (Insel et al., 2010). For research purposes, the use of a continuous score provides more power and allows the application of more advanced statistical methods. Multiple studies have demonstrated that such a dimensional approach can further contribute to a better etiological understanding of attention-deficit/hyperactivity problems (Hudziak, Achenbach, Althoff, & Pine, 2007; Lubke, Hudziak, Derks, van Bijsterveldt, & Boomsma, 2009; Polderman et al., 2007; Shaw et al., 2011). However, despite these insights and developments in the clinical field, the majority of (neurobiological) research in psychiatry continues to be performed in a case-control design or in solely clinical samples. In clinical samples, symptoms are usually more severe and there is a higher chance of referral bias by impaired children, thereby limiting the generalizability of findings to a broader population. By also studying problem behavior such as attention and hyperactivity problems in the general population (by focusing on population-based cohorts of children) we might gain a better understanding of the continuum of psychopathology and its etiology in developmental psychiatric disorders like ADHD. Furthermore, doing so would make it possible to extend previously drawn conclusions based on clinical samples to the full range of problems in the general population.

Neurobiology of ADHD symptoms

Although ADHD is one of the most common neurodevelopmental disorders, relatively little is known about the underlying neurobiology. Recent genetic studies have shown that, next to environmental factors, genetic factors play a major role in the development of ADHD, with heritability estimates around 70% (Faraone et al., 2005; Nikolas & Burt, 2010; Posthuma & Polderman, 2013). ADHD is thought to be polygenic, implying that many genes, each having a very small individual effect (and possibly interacting with each other and environmental factors), are implicated. In addition to this, the clinical

presentation of ADHD is highly heterogeneous, as not all children exhibit the same set of problems and the same degree of severity. Largely because of this complex nature of the disorder, genome-wide association studies (GWAS) that have been performed over the last decade have not been successful in identifying the putative genes responsible for ADHD (Neale et al., 2010). Therefore, new approaches are currently being sought to tackle the polygenic and heterogeneous nature of ADHD, including studying gene-sets consisting of multiple (functionally) related genes, instead of single genes or single-nucleotide polymorphisms (SNPs). Such analyses generally increase power, as they reduce multiple testing (compared to testing multiple separate SNPs or genes) and improve the interpretability of findings (Lips et al., 2012; Wang, Li, & Bucan, 2007).

At the same time, neuroimaging studies have shown mixed findings with regard to brain structure and function in ADHD. Among cortical findings, a widespread thinner cortex (Ducharme et al., 2012; Narr et al., 2009) and a delay in brain maturation (Shaw et al., 2007) have been mentioned, as well as aberrant gyrification (the amount of folding of the brain) (Wolosin, Richardson, Hennessey, Denckla, & Mostofsky, 2009). Among subcortical findings, one of the most consistent abnormalities is a reduction in volume of the basal ganglia (Frodl & Skokauskas, 2012; Nakao, Radua, Rubia, & Mataix-Cols, 2011; Shaw et al., 2014). However, as mentioned, results have been mixed and since the large majority of these studies was performed in clinically referred samples, population-based studies assessing the neurobiology of attention and hyperactivity problems along a continuum are lacking.

Cognitive problems associated with ADHD symptoms

In addition to behavioral problems, deficits in cognitive functioning are commonly reported in ADHD. Studies have shown moderate correlations between ADHD symptom scores and IQ scores and an on average 9 point lower mean IQ in children with a diagnosis of ADHD (Frazier, Demaree, & Youngstrom, 2004; Kuntsi et al., 2004). However, it is unclear whether this lower IQ represents a general cognitive problem, or actually reflects deficits in more specific cognitive domains. In order to parse out the specific cognitive problems, multiple studies have tested neuropsychological performance in clinical ADHD samples (Brodsky, Willcutt, Davalos, & Ross, 2014; Frazier et al., 2004; Willcutt, Sonuga-Barke, Nigg, & Sergeant, 2008). Although results of these studies are mixed, two large meta-analyses (Frazier et al., 2004; Willcutt et al., 2008) have suggested that ADHD appears to be most strongly related to tasks assessing executive functioning (EF). In addition to the question of specificity of cognitive problems in ADHD, the question arises whether these cognitive problems (whether specific or global) are also found in the general population, when studying attention and hyperactivity problems along a continuum. To our knowledge, no studies have been performed assessing the relation between continuously measured attention-deficit/hyperactivity problems and cognitive functioning in large population-based samples of young children.

In the current DSM-5 classification system (American Psychiatric Association, 2013), cognitive problems are not regarded a criterion for the diagnosis of ADHD, but are rather seen as a comorbid problem. However, twin studies have reported a shared genetic etiology of cognitive ability and ADHD (Jacobs et al., 2002; Kuntsi et al., 2004; Polderman et al., 2009; Polderman et al., 2006). This shared genetic background suggests that a common neurobiology, which could for example be reflected in brain morphology, underlies ADHD and problems in cognitive functioning. If such a common underlying neurobiology indeed exists, this would suggest that cognitive problems in ADHD should not be seen as a separate comorbid problem, but are actually an integral part of the disorder.

Normal development of cognition

In order to study and understand aberrant cognitive development, one should be familiar with the typical development of cognitive ability in children. Awareness of age- and gender-related differences in cognitive development is essential for a correct understanding of problems in cognitive development. Besides, since early childhood is a period of major neurocognitive development (Casey, Tottenham, Liston, & Durston, 2005; Giedd et al., 1999; Gogtay et al., 2004), studying typical neuropsychological functioning already at a young age is very important. Previous results with regard to the age of maturation of several specific aspects of functioning (such as language, memory, executive functioning, visuospatial processing and sensory-motor functions) have been mixed (Anderson, Anderson, Northam, Jacobs, & Catroppa, 2001; Del Giudice et al., 2000; Huizinga, Dolan, & van der Molen, 2006; Korkman, Barron-Linnankoski, & Lahti-Nuuttila, 1999; Korkman, Kemp, & Kirk, 2001; Korkman, Lahti-Nuuttila, Laasonen, Kemp, & Holdnack, 2013; Rosselli, Ardila, Navarrete, & Matute, 2010). Furthermore, it is known that boys and girls have slight differences in their cognitive development. It is generally said that on average girls outperform boys on language and other verbal tasks, while boys tend to perform better on tasks that require spatial abilities, but contrasting results have been reported as well (Ardila, Rosselli, Matute, & Inozemtseva, 2011; Mann, Sasanuma, Sakuma, & Masaki, 1990; Strand, Deary, & Smith, 2006; Voyer, 2011).

One feature of the brain that may underlie cognitive ability is structural connectivity. As efficient communication between different interacting brain regions is important for cognitive functioning, white matter integrity (a measure of structural connectivity) has been previously studied in relation to cognitive ability (Erus et al., 2015; Fryer et al., 2008; Johansen-Berg, Della-Maggiore, Behrens, Smith, & Paus, 2007; Muetzel et al., 2008; Navas-Sanchez et al., 2014; Schmithorst, Wilke, Dardzinski, & Holland, 2002). These studies have found white matter integrity to be positively related to cognitive functioning. However, studies assessing the relationship between white matter integrity and cognitive ability in a general population sample of young children, and especially with large sample sizes, remain limited. In addition, as different cognitive functions mature at different ages, and as different brain regions regulate these cognitive functions, it would be informative to also study the association between white matter integrity and a broad range of specific neuropsychological domains.

Aims

In this thesis, the neurobiology and neuropsychology of attention-deficit/hyperactivity problems is studied. The majority of the studies described in this thesis are performed in a large population-based cohort of young children between six and nine years of age. By using a population-based sample and continuous scores of inattention and hyperactivity/impulsivity (rather than dichotomizing children into groups), our study covers the entire spectrum of attention-deficit/hyperactivity problems, and thus includes both children with no or very little problems as well as children with clinical problems. This provides greater generalizability with the general population as a whole, compared to study samples recruited from a clinical setting, and makes it possible to extend previously drawn conclusions based on clinical samples to the full range of problems in the general population. Furthermore, it provides important insights with regard to the expected dimensionality of the underlying neurobiology of attention and hyperactivity problems.

The goals of this thesis were 1) to explore the neurobiology (imaging and genetics) of attention-deficit/hyperactivity problems, 2) to study the normal development of cognitive ability, in order to 3) study cognitive problems associated with attention-deficit/hyperactivity problems.

Setting

All but one study described in this thesis were embedded within the Generation R Study. The study described in chapter 4 was performed in a different sample. The children of this study were recruited as part of a neuroimaging study of the University of Minnesota Medical School. All children were between nine and nineteen years of age and had either a clinical diagnosis of ADHD (n=19) or VCFS (n=9) or were normal controls (n=23).

The remaining 5 chapters were performed in a subsample of the Generation R Study cohort. The Generation R Study is a large prospective population-based cohort study in Rotterdam (the Netherlands) that investigates children's health, growth and development from fetal life onwards (Jaddoe et al., 2012; Tiemeier et al., 2012). All pregnant women living in Rotterdam with an expected delivery date between April 2002 and January 2006 were invited to participate. In total, 9,778 women participated in the study. During the study, child, parental, and environmental characteristics were collected through questionnaires. When the children were between 5 and 7 years of age, more detailed hands-on assessments were done in all children that were participating in that stage (n=8,305). In this same period, a brain Magnetic Resonance Imaging (MRI) study began within a subsample of the Generation R Study (White et al., 2013). Between September 2009 and July 2013, a total of 1,325 six-to-nine year-old children were recruited for this imaging study. Of this group, 1,070 children were scanned, 1,307 children underwent an extensive neuropsychological assessment and a total of 1,053 children had a combination of both imaging and neuropsychological data available.

Outline

Chapter 2 describes the association between cortical thickness, a measure of brain morphology, and inattention/hyperactivity symptoms. In chapter 3, a combination of neuroimaging and genetics is used to study the neurobiology of attention-deficit/hyperactivity problems. In this chapter we study the role of candidate genetic pathways and volume of basal ganglia structures. In chapter 4, we compare patterns of brain gyrification between two groups of children that both exhibit attention and hyperactivity problems; namely children with a diagnosis of ADHD and children with velocardiofacial syndrome (VCFS). Chapter 5 describes normal development of cognitive ability, by investigating the association of age, gender and intelligence with neuropsychological functioning in typically developing children. In connection to this study, chapter 6 focuses on the relation between white matter integrity and neuropsychological functioning. In chapter 7, we study cortical morphology (cortical thickness and gyrification) as potential shared neurobiological substrate underlying both attention-deficit/hyperactivity problems and cognitive ability. Finally, in chapter 8, the main findings of these studies, methodological considerations, and implications for clinical practice and future research are discussed.

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CHAPTER

2

Cortical thickness and inattention/ hyperactivity symptoms in young children

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Published as Psychological Medicine 2014, 44(15), 3203-3213

ABSTRACT

Background

While many neuroimaging studies have investigated the neurobiological basis of Attention-Deficit/Hyperactivity Disorder (ADHD), few have studied the neurobiology of attention problems in the general population. The ability to pay attention falls along a continuum within the population, with children with ADHD at one extreme of the spectrum and therefore, a dimensional perspective of evaluating attention problems has an added value to the existing literature. Our goal was to investigate the relationship between cortical thickness and inattention and hyperactivity symptoms in a large population of young children.

Methods

This study is embedded within the Generation R Study and includes 444 six-to-eight year-old children with parent-reported attention and hyperactivity measures and high-resolution structural imaging data. We investigated the relationship between cortical thickness across the entire brain and the Child Behavior Checklist Attention Deficit/Hyperactivity Problems score.

Results

We found that greater attention problems and hyperactivity were associated with a thinner right and left postcentral gyrus. When correcting for potential confounding factors and multiple testing, these associations remained significant.

Conclusions

In a large, population-based sample we showed that young (six-to-eight year-old) children who show more attention problems and hyperactivity have a thinner cortex in the region of the right and left postcentral gyrus. The postcentral gyrus, being the primary somatosensory cortex, reaches its peak growth early in development. Therefore, the thinner cortex in this region may reflect either a deviation in cortical maturation or a failure to reach the same peak cortical thickness compared to children without attention or hyperactivity problems.

INTRODUCTION

While attention problems are one of the core characteristics of Attention-Deficit/Hyperactivity Disorder (ADHD), the ability to pay attention falls along a continuum within the population and children with ADHD are at one extreme of the spectrum (Polderman et al., 2007, Lubke et al., 2009). In addition, attention problems are commonly found in other childhood psychiatric disorders, such as early-onset psychoses and pervasive developmental disorders (Swaab-Barneveld et al., 2000, Muratori et al., 2005, Karatekin et al., 2010, van Rijn et al., 2012). There has been considerable debate recently over whether child psychopathology falls within diagnostic categories with clearly defined boundaries, or whether symptoms could be better described within a dimensional (continuous) framework. Numerous studies provide evidence in favor of a dimensional approach, as it provides greater statistical power and contributes an additional perspective to the existing literature (Hudziak et al., 2007, Polderman et al., 2007, Lubke et al., 2009, Shaw et al., 2011). Furthermore, dimensional approaches are being evaluated as a part of the National Institutes of Mental Health's Research Domain Criteria (Insel et al., 2010).

While many neuroimaging studies have investigated the neurobiological basis of ADHD (reviewed in Durston, 2003), few studies have evaluated the underlying neurobiology of attention problems from a dimensional perspective. Shaw et al. (2011) studied cortical thickness in a non-clinical sample of 193 typically developing youth, as well as in a clinical sample of 197 children with ADHD (both 8-18 years of age). They found that the rate of cortical thinning changed gradually with the degree of symptom severity; youth with higher levels of hyperactivity and impulsivity in the non-clinical sample had a slower rate of cortical thinning and children with a clinical ADHD diagnosis showed the slowest rate of cortical thinning (Shaw et al., 2011). Additionally, a recent study by Ducharme et al. (2012) evaluated the association between cortical thickness and attention problem scores in a sample of healthy children between 6 and 18 years of age. Their findings demonstrated an association between increased attention problem scores and specific regions with a thinner cortex, as well as slower cortical thinning with aging in different areas involved in attention processes (Ducharme et al., 2012).

Studies using clinical samples to examine cortical thickness in children with ADHD have shown a highly significant thinner cortex over wide areas of the brain, implicating a thinner cortex to be an important marker for ADHD (Narr et al., 2009). In a large longitudinal study of children and adolescents with ADHD, Shaw et al. (2006) also showed that children with ADHD have significantly thinner cortices across the entire brain (Shaw et al., 2006). In a subsequent study, Shaw et al. (2007) found that, although the overall pattern of cortical development was similar in children with ADHD and controls, the trajectories of cortical thinning were different. They reported that children with ADHD were delayed in attaining peak cortical thickness throughout most of the cerebrum. The only region in which they found the ADHD group to show slightly earlier maturation was the sensorimotor region (Shaw et al., 2007). The increase in cortical thickness during normal development may be driven by mechanisms like dendritic spine growth and the expansion of supporting glia (Chklovskii et al., 2004, Sur and Rubenstein, 2005). The cortical thinning that follows may reflect intracortical myelination and the creation of efficient neural networks (by the elimination of unused synapses), including those networks that support cognition (Huttenlocher and Dabholkar, 1997, Hensch, 2004). The delayed maturation and later cortical thinning in ADHD may therefore point to less efficient brain networks, possibly causing the cognitive and behavioral difficulties that children with ADHD experience.

Because of the lack of studies focusing on attention problems in general population samples of children and the recent tendency towards favouring a dimensional perspective of child psychopathology, the goal of our study was to investigate the relationship between cortical thickness and inattention/hyperactivity symptoms along a continuum in a very large, population-based sample of young children. By including a large sample of children within a narrow age range, our goal was to obtain a clear snapshot of this relationship during a very specific period of child neurodevelopment. Furthermore, the recruitment of children from a large longitudinal prenatal population-based cohort study of child development provides the ability to assess multiple potential confounding factors and is more representative of the population at large.

MATERIALS AND METHODS

Participants

This study is embedded within the Generation R Study, a multi-ethnic population-based cohort study, investigating children's health, growth and development from fetal life until young adulthood in Rotterdam, the Netherlands. An overview of the Generation R Study design and population is described elsewhere (Jaddoe et al., 2012, Tiemeier et al., 2012).

A total number of 8,305 children participated in the study phase from 5 to 16 years (Jaddoe et al., 2012). At age 6 years, a pilot brain Magnetic Resonance Imaging (MRI) study began within the Generation R Study. An overview of this neuroimaging component of the Generation R Study and participant selection is provided elsewhere (White et al., 2013). A total of 608 six-to-eight year-old children were scanned between September 2009 and February 2012. Of the 608 children with imaging data, a total of 104 children were excluded based upon poor image quality. For the children with good quality imaging data, data on attention problems were missing in 45 children. Furthermore, data were collected on 6 twin pairs and 3 sibling pairs. Twin pairs were excluded from the analyses, as well as a randomly selected child from each sibling pair. This resulted in a final study sample of 444 children.

Covariates

In Table 2.1, participant characteristics are presented. To define child ethnicity, the ethnicity categorization of Statistics Netherlands (Statistics Netherlands, 2004a) was used. Children with both parents born in the Netherlands were considered Dutch and children were classified as non-Dutch (further categorized as 'other Western', 'Turkish/Moroccan', 'Surinamese/Antillean' or 'other non-Western') if one parent was born outside the Netherlands. Maternal education was defined as highest education completed, according to the definition of Statistics Netherlands (Statistics Netherlands, 2004b) and household income was defined by the total net monthly income of the household. Information on maternal alcohol use and smoking during pregnancy was obtained using questionnaires in each trimester of pregnancy. Information on the date of birth, gender, and birth weight was obtained from midwives and hospital registries. Gestational age was established using ultrasound measures during pregnancy. IQ of the child was assessed during the assessment wave at 6 years of age, using a shortened version of the Snijders-Oomen Niet-verbale intelligentie Test – Revisie (SON-R 2.5–7), which is a nonverbal intelligence test suited for children of 2.5–7 years of age (Tellegen et al., 2005). Handedness of the child was

obtained using the Edinburgh Handedness Inventory (Oldfield, 1971) on the day of the scan, as well as information regarding the use of psychostimulant medication.

Child Behavior Checklist

During the assessment wave at 6 years of age, all parents were asked to fill out the Child Behavior Checklist (CBCL) 1½ - 5 (Achenbach and Rescorla, 2000). The preschool CBCL was chosen because many children were younger than 6 years at the time of the assessment and older age versions are inappropriate for such young children (as they contain questions on for example tobacco smoking and the use of other substances). The use of one version of the CBCL was desired, in order to enhance comparability between all children. In the CBCL 1½ - 5, the primary caregiver is asked to answer 99 items as 0 for not true, 1 for somewhat or sometimes true, and 2 for very true or often true, on the behavior of their child in the preceding two months. Good reliability and validity have been reported for the preschool version of the CBCL (Achenbach and Rescorla, 2000). To measure inattention and hyperactivity, we used the raw sumscore of the DSM-oriented Attention Deficit/Hyperactivity Problems (ADHP) scale. The ADHP scale measures attention problems and symptoms of hyperactivity. Cronbach's alphas were similar in the 5-year-old children and in children of 6 years and older for the ADHP scale (α=0.83 and α=0.86 respectively), indicating that the attention and hyperactivity problems were reliably measured in the children older than 5 years of age. The primary caregiver completed the CBCL, this was the mother in 93.5% of the cases.

Imaging

MR images were acquired using a GE Discovery MR750 3.0 Tesla scanner (GE Healthcare Worldwide, Milwaukee, WI, USA) with an 8-channel head coil. The high-resolution T1-weighted image was collected using an inversion recovery fast spoiled gradient recalled (IR-FSPGR) sequence with the following parameters: TR = 10.3 ms, TE = 4.2 ms, TI = 350 ms, NEX = 1, flip angle = 16°, readout bandwidth= 20.8 kHz, matrix 256 x 256, imaging acceleration factor of 2, and an isotropic resolution of 0.9x0.9x0.9 mm^3. Before scanning took place, children were familiarized with the scanning environment during a mock scanning session. All procedures have been described in detail elsewhere (White et al., 2013).

The study was approved by the Medical Ethics Committee (METC) of the Erasmus Medical Center and the Central Committee of Research involving Human Subjects (CCMO). Written informed consent was obtained from the parents of all participants.

Image Quality

In the 608 children with imaging data, we performed image quality assurance in 2 steps. The first step was a visual inspection of the image quality of the T1 sequence prior to preprocessing the data. All images were rated on a 6-point scale (unusable, poor, fairly good, good, very good, excellent). The next step of quality assurance took place after the images were processed through the FreeSurfer pipeline, and consisted of a visual inspection of the segmentation quality of the data. All images were rated on a 7-point scale (not constructed, poor, fair, fairly good, good, very good, excellent). T1 data that were rated as unusable or poor were not used (n=34), as well as the children whose FreeSurfer output was not constructed or rated as poor (n=70), leading to a total of 104 children that were excluded based upon poor image quality (i.e., excessive movement or other artifacts). In the total sample of 608 children with

structural imaging data, we utilized a $\chi 2$ analysis to evaluate if there was a relationship between image quality and attention problems. We found no differences in image quality between children with more or less attention problems and/or hyperactivity.

Image Processing

Cortical reconstruction and volumetric segmentation was performed with the FreeSurfer image analysis suite (http://surfer.nmr.mgh.harvard.edu/), version 5.1. The technical details of these procedures are described in prior publications (Dale et al., 1999, Jovicich et al., 2006, Reuter et al., 2012). Cortical thickness was calculated as the closest distance from the gray/white boundary to the gray/CSF boundary at each vertex on the tessellated surface (Fischl and Dale, 2000). The surface based map was smoothed using a 10 mm full-width half-maximum (FWHM) Gaussian kernel prior to the surface based analyses. Procedures for the measurement of cortical thickness have been validated against histological analysis (Rosas et al., 2002) and manual measurements (Kuperberg et al., 2003, Salat et al., 2004). FreeSurfer morphometric procedures have been demonstrated to show good test-retest reliability across scanner manufacturers and across field strengths (Han et al., 2006). Numerous studies using FreeSurfer in typical and atypical developing school-age children are available (O'Donnell et al., 2005, Derauf et al., 2009, Ghosh et al., 2010, Ducharme et al., 2012, Juuhl-Langseth et al., 2012, Webb et al., 2012, Yang et al., 2012).

TABLE 2.1. **PARTICIPANT CHARACTERISTICS (n=444)**

	MEAN (SD)[a]
CHILD CHARACTERISTICS	
Gender (% boys)	51.8
Ethnicity (%)	
• Dutch	65.8
• Other Western	8.1
• Turkish or Moroccan	9.2
• Surinamese or Antillean	9.0
• Other Non-Western	7.9
Gestational age at birth, weeks	40.0 (1.7)
Birth weight, grams	3455 (542)
CBCL score Attention Problems scale	2.02 (2.12)
• range	0 - 9
CBCL score Attention Deficit/Hyperactivity Problems scale	3.75 (3.12)
• range	0 - 12
IQ	102.01 (14.15)
Handedness (% right)	89.4
Age CBCL, years	6.13 (0.41)
• range	5.25 – 7.92
Age MRI, year	7.70 (0.92)
• range	6.13 – 9.61
Time interval between CBCL and MRI, years	1.6 (0.84)
Psychostimulant use (%)[b]	
• Yes	3.6
• No	89.6
MATERNAL CHARACTERISTICS	
Education level (%)	
• High	47.1
• Medium	38.7
• Low	14.2
Monthly household income (%)	
• >2000	72.5
• 1200-2000	20.3
• <1200	7.2
Alcohol use during pregnancy (%)	
• Never	38.1
• Until pregnancy was known	12.8
• Continued during pregnancy	49.1
Smoking during pregnancy (%)	
• Never	78.4
• Until pregnancy was known	3.8
• Continued during pregnancy	17.8

NOTE. [a] Values given as mean and standard deviation, unless otherwise indicated. [b] Data regarding psychostimulant use missing in n=30. CBCL = Child Behavior Checklist 1.5-5; IQ = Intelligence Quotient; MRI = Magnetic Resonance Imaging.

Statistical analyses

As the boundaries of a priori-defined regions of interest may not exactly overlap the boundaries of the actual areas in which abnormalities are located, we chose to perform vertex-wise exploratory analyses of cortical thickness across the entire brain. To investigate the relationship between cortical thickness and inattention and hyperactivity symptoms, we performed surface-based General Linear Model (GLM) vertex-wise cortical analyses using the FreeSurfer in-built module QDEC (www.surfer.nmr.mgh.harvard.edu). QDEC allows users to perform inter-subject/group averaging and inference on the morphometry data produced by the FreeSurfer processing stream. We ran QDEC to investigate the correlation between cortical thickness on vertices covering the entire cortex and the CBCL ADHP score. Age during scanning and gender were included as covariates in the analysis. To correct for multiple testing (for all brain vertices), a Monte Carlo Null-Z Simulation was performed, using a threshold of 1.3 ($p<0.05$). Monte Carlo Null-Z Simulation is a cluster-wise correction and controls the rate of false positive clusters (method based on Hagler et al., 2006). Monte Carlo corrected p-values are reported.

Cortical thickness data of significant cluster(s) identified in the vertex-wise QDEC analyses were extracted for each individual and imported into SPSS (version 20.0) for further detailed analyses. Using these extracted cortical thickness measures, we performed linear regression analyses with cortical thickness of the cluster(s) (residualized for age during scanning) as the independent variable and the ADHP score as the dependent variable. These analyses were performed correcting for other, possibly confounding, factors that could not be corrected for using QDEC, given constraints on the model setup. In this way we evaluated whether the association(s) would remain present after correcting for other, possibly important, factors. Regression analyses were corrected for gender and age when the CBCL was completed, other possibly important variables were considered confounders and were added to the regression analyses only when they changed the effect estimate (B) by 5% or more. These included ethnicity, IQ, and maternal smoking during pregnancy.

In all analyses, missing values of potential confounding environmental or risk factors (0.2% for handedness, 7.4% for IQ, 2.0% for maternal education, 4.8% for household income, 4.6% for alcohol use during pregnancy and 1.5% for smoking during pregnancy) were imputed using the multiple imputation (Markov chain Monte Carlo) method in SPSS 20.0 with 5 imputations and 10 iterations. In all analyses, CBCL scores were square root transformed to approach a normal distribution.

RESULTS

Vertex-wise analyses

The results of the initial vertex-wise QDEC analyses are presented in Table 2.2 and Figure 2.1. After correcting for multiple testing, we observed a significant cluster in the right and left postcentral gyrus. We found thickness of the right ($p=0.0001$) and left ($p=0.01$) postcentral gyrus to be negatively correlated with the CBCL ADHP score, indicating a thinner cortex in relation to higher ADHP scores.

TABLE 2.2. **QDEC CORRELATION CORTICAL THICKNESS AND CBCL ADHP SCORE[a,b]**

LOCATION	CLUSTER SIZE (MM^2)	TALAIRACH COORDINATES			NO. OF VERTICES WITHIN CLUSTER	CLUSTERWISE P-VALUE[a]	PEAK P-VALUE WITHIN CLUSTER
		TALX	TALY	TALZ			
Postcentral (RH)	3013.0	29.3	-40.7	57.9	7223	0.00010	0.00006
Postcentral (LH)	1009.2	-30.0	-28.9	63.4	2283	0.01160	0.00030

NOTE. CBCL = Child Behavior Checklist 1.5-5; ADHP = Attention Deficit/Hyperactivity Problems. RH = right hemisphere; LH = left hemisphere. TalX = Talairach region X plane; TalY = Talairach region Y plane; TalZ = Talairach region Z plane. [a] Monte Carlo Simulation ($p < 0.05$) applied to correct for multiple testing. [b] Analyses accounting for gender. Age during scanning used as nuisance factor.

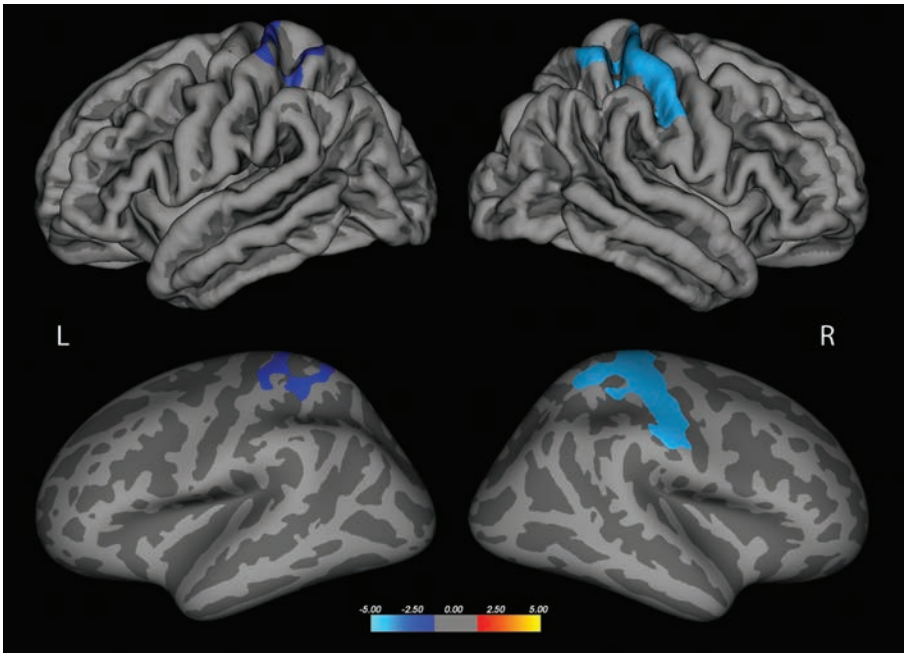

FIGURE 2.1. Statistical maps of the significant clusters in the left and right hemispheres for the Child Behavior Checklist Attention Deficit/Hyperactivity Problems Scale, represented on both the pial (top) and inflated (bottom) surfaces. Monte Carlo Simulation was applied to correct for multiple testing. Colors represent the -log10(p-value), the blue (negative) cluster equals a negative relationship between cortical thickness and CBCL score. L = left hemisphere; R = right hemisphere.

Detailed analyses of clusters

Using the extracted cortical thickness data from both clusters for each individual, we first calculated bivariate correlations between cortical thickness of the identified clusters and the CBCL ADHP score. We observed significant (all $p<0.01$) negative correlations between on the one hand the thickness of the right postcentral ADHP cluster and the left postcentral ADHP cluster and on the other hand the CBCL score (respectively $r(442)=-0.26$ and $r(442)=-0.22$), such that a thinner cortex was associated with more attention problems and hyperactivity.

We then performed linear regression analyses in SPSS, while correcting for potential confounders (age when CBCL was completed, gender, ethnicity, IQ and maternal smoking during pregnancy) and with cortical thickness residualized for age during scanning (Table 2.3). For the adjusted model with the additional covariates, the ADHP score showed a significant association with the thickness of the right postcentral cluster (B=-1.24, p<0.001) and the thickness of the left postcentral gyrus (B=-0.95, p<0.001). When excluding children that used psychostimulant medication (or that had missing data regarding medication use) all results remained the same.

To rule out a potential confounding effect of other comorbid behavioral or emotional problems, we additionally adjusted the analyses for the other CBCL DSM-oriented scale scores (Affective Problems, Anxiety Problems, Pervasive Developmental Problems and Oppositional Defiant Problems). The results remained similar. We also additionally adjusted the analyses for scan quality, to rule out a potential confounding effect of scan quality on the association between cortical thickness and CBCL attention and hyperactivity problems. These analyses again yielded similar results.

TABLE 2.3. **SPSS REGRESSION ANALYSES THICKNESS CLUSTERS AND CBCL ADHP SCORE**

	MODEL 1		MODEL 2 (ADJUSTED)	
	B (95% CI)	P	B (95% CI)	P
Postcentral cluster thickness (RH)	-1.36 (-1.85;-0.87)	<0.001	-1.24 (-1.72;-0.75)	<0.001
Postcentral cluster thickness (LH)	-1.06 (-1.51;-0.61)	<0.001	-0.95 (-1.40;-0.50)	<0.001

NOTE. CBCL = Child Behavior Checklist 1.5-5; ADHP = Attention Deficit/Hyperactivity Problems. RH = right hemisphere; LH = left hemisphere. Cortical thickness was residualized for age during scanning. Model 1 only adjusted for gender and age when CBCL was completed. Model 2 additionally adjusted for ethnicity, child IQ and maternal smoking during pregnancy. The B's are not interpretable since mathematically transformed scores were used in the analyses.

DISCUSSION

In a large population-based group of six-to-eight year-old children, we found that cortical thickness in the region surrounding the postcentral gyrus was significantly negatively associated with symptoms of inattention and hyperactivity. A thinner cortex in this region was related to a higher CBCL inattention and hyperactivity score. The relation remained present after adjusting for several confounding factors, including gender, age, ethnicity, IQ, and maternal smoking.

The postcentral gyrus is a structure of the parietal lobe where the primary somatosensory cortex is located (Brodmann areas 1 through 3). The cluster also extends into the somatosensory association cortex (Brodmann area 5). Earlier studies of cortical mapping of motor and sensory areas of the human cortex showed that there is a considerable overlap between the motor and sensory areas of the brain. There appears to be considerable functional heterogeneity of the precentral and postcentral areas, with approximately 25% of all motor activations located postcentral. This indicates that the human motor and sensory areas have no exact boundaries and are not simply divided by the central sulcus (Penfield and Boldrey, 1937, Nii et al., 1996), which implicates that the clusters found in our study are potentially involved in both sensory and motor functioning.

Somatosensory processing plays an important role in typical development and has been found to be disturbed in various neurodevelopmental disorders. It is known that the development of motor skills depends heavily on the somatosensory system and touch also plays an important role in social and communication skills in early childhood and beyond. Neurodevelopmental disorders are characterized by behavioural, emotional, motor, or cognitive problems, and touch plays a role in all of these areas (Cascio, 2010). Interestingly, it has been shown that brain responses to somatosensory stimuli are aberrant in children with ADHD (Parush et al., 2007), possibly suggesting a deficit in the perception-to-action system of the brain (Dockstader et al., 2009).

Shaw et al. (2007) showed that children with clinical ADHD were delayed in attaining peak cortical thickness throughout most of the cerebrum, except for the sensorimotor area, which seems to be maturing earlier. After reaching peak cortical thickness, cortical thickness declines in both typically developing children and those with ADHD. For the sensorimotor region peak cortical thickness is reached at approximately 7.0 years of age in ADHD and 7.4 years of age in healthy controls (Shaw et al., 2007). The mean age of the children in our group was 7.7 years, which is approximately the expected period of peak cortical thickness in this region. Therefore, the thinner cortex in the somatosensory region in our study either suggests that the peak cortical thickness is less in children with attention problems and hyperactivity or alternatively, may point to a deviation in the developmental trajectory of cortical thickness. This deviation could either represent earlier thinning, as was shown by Shaw et al. (2007), or a delay in reaching peak cortical thickness. Additional measurement points will be necessary to model trajectories of cortical thickness.

In contrast to our findings, two previous studies on cortical thickness and attention problems in a broader sense (Ducharme et al., 2012, Walhovd et al., 2012) did not find a direct association between attention problem scores and cortical thickness. However, Ducharme et al. (2012) found (in a sample of 257 children) an 'attention problems by age' interaction with cortical thickness. According to the authors, they did not find a direct relationship because of a disappearance of the negative association between attention problems and cortical thickness with age. Since we, in contrast to Ducharme et al. (2012), studied a very small age range, this may explain why we did find a direct association between greater attention problems and cortical thickness. In addition, the study of Ducharme et al. (2012) included only healthy children. In their study all children had a CBCL Attention Problems t-score below 70, which is the clinical cut-off, whereas we used a population-based sample that included children with clinically elevated CBCL scores (3.2% of our sample had a t-score at or above 70). This may also explain the discrepancy between the findings, as it might be more difficult to find an association in a population that is free of clinically affected persons. In addition, our large study sample and narrow age range provides greater power to detect a direct association between cortical thickness and attention problems in a population-based sample. Another study that did not find an association between cortical thickness and attention problems, is a study by Wolosin et al. (2009). The discrepancy between our findings and theirs might be explained by a lack of power in the study of Wolosin et al. (2009), since their study sample consisted of only 56 children (Wolosin et al., 2009). Another difference between the two studies is that Wolosin et al. (2009) compared children with a clinical diagnosis of ADHD (21 children) and healthy controls (35 children), whereas we studied attention problems along a continuum. Furthermore, the age range of their sample is different, as they studied children between the ages of 8 and 12. The combination of these differences and a lack of power, might possibly explain the discrepancy in findings between the studies.

Shaw et al. (2011) also examined the relationship between hyperactivity/impulsivity and cortical thickness in a sample of 193 typically developing children (and 197 children with ADHD). Interestingly, they found the rate of cortical thinning to be slower in children with higher levels of hyperactivity/impulsivity in the region surrounding the supplementary motor area, extending into the region located in our study (Shaw et al., 2011). While they also found other regions implicated, their sample included a longitudinal design with a much broader age range, which allowed them to assess trajectories as well as differences. Since the children in our study fall within a very narrow age range, our results represent a very specific neurodevelopmental period.

Studies of typically developing children have shown a characteristic temporal progression within regions of brain development. In a longitudinal study of healthy children, Gogtay et al. (2004) showed that the primary sensorimotor cortices mature first, together with the frontal and occipital poles of the cortex. Maturation then progresses in a parietal to frontal wave of development (Gogtay et al., 2004). Since the children of our study are young, it is not surprising that we found differences in brain regions that have been shown to be the first to mature. As other brain regions, such as prefrontal areas, are still developing in these young children, it is possible that cortical thickness deviations in these regions will emerge later as the neurodevelopmental differences become unmasked. This hypothesis is in line with previous work of Shaw et al. (2007) in a sample of both children and adolescents with ADHD. In this older sample the authors showed a deviation in attaining peak cortical thickness in other parts of the brain as well, such as prefrontal regions (Shaw et al., 2007). In addition, in a study on cortical thickness in adults with ADHD, exploratory analyses showed a thinner cortex in adult ADHD in multiple brain regions, including a cluster in the left sensorimotor region, although these findings did not survive the stringent correction for multiple testing (Makris et al., 2007). However, to actually test our hypothesis on the potential later emergence of deviations in cortical thickness in regions that mature later in development, longitudinal studies that also include older children and adolescents will be needed.

An important strength of our study is the very large sample size and narrow age range, which provided us with greater power to detect differences than previous studies (Ducharme et al., 2012, Walhovd et al., 2012). In addition, the small age range allowed us to evaluate cortical morphology during a very specific window of development. Since neurodevelopment in young children is still ongoing, a larger age range may result in age-dependent differences that dilute or mask the findings, as pointed out in the study of Ducharme et al. (2012). Another strength is the young age and narrow age range of the children, since ADHD is often diagnosed in school age children and our study provides a snapshot of brain development at a period closer to this age. Furthermore, few studies on cortical morphology have been performed in a large group of children this young. Additional strengths of the study include the population-based design, which provides greater generalizability with the population. Finally, tapping a prenatal longitudinal cohort study provides a wealth of information covering numerous environmental and other risk factors that can be used to control for potential confounding factors in the relationship between cortical thickness and attention problems and hyperactivity.

A limitation of our study is that the neuroimaging was performed at only one time point. Therefore, no inferences can be made on causality (direction of effect) or trajectories of neurodevelopment. Also, the neuroimaging and the collection of the CBCL data were done at different time points. The mean time interval between the collection of the CBCL and the neuroimaging was 1.6 years. Although CBCL ADHP scores have been shown to have high stability over time in both clinical (Stanger et al., 1996,

Biederman et al., 2001) and population-based samples (McConaughy et al., 1992, Verhulst and van der Ende, 1992), this may influence the results. To try to account for this, we controlled for both age when CBCL was completed and the age during scanning. Finally, due to the lack of a suitable child atlas, we used an adult atlas within FreeSurfer for segmentation of the images. However, as noted before, numerous studies in both typical and atypical developing children have used FreeSurfer successfully (O'Donnell et al., 2005, Derauf et al., 2009, Ghosh et al., 2010, Ducharme et al., 2012, Juuhl-Langseth et al., 2012, Webb et al., 2012, Yang et al., 2012).

To conclude, we demonstrated in a large, population-based sample that young (six-to-eight year-old) children who show more attention problems and hyperactivity have a thinner cortex in the region of the right and left somatosensory cortex. Since there is evidence that cortical gray matter in this region peaks during this age range, the thinner cortex in this region may reflect either a decrease in peak cortical thickness in children with more attention problems and hyperactivity, or alternatively, a deviation in cortical maturation. Longitudinal studies starting in young children will be important to better understand the growth trajectories of cortical thickness in children with attention and hyperactivity problems. Our finding of a thinner cortex in a population-based sample of children showing attention problems and hyperactivity also provides support for the dimensional aspect of attention and hyperactivity problems in children.

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CHAPTER

3

Imaging genetics of inattention and hyperactivity/ impulsivity symptoms: basal ganglia and genetic pathways

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Journal of the American Academy of Child & Adolescent Psychiatry, accepted for publication

ABSTRACT

Background

Although attention-deficit/hyperactivity disorder (ADHD) is one of the most common neurodevelopmental disorders, little is known about the neurobiology. Clinical studies have suggested morphology of the basal ganglia to play a role. Furthermore, symptoms of hyperactivity/impulsivity have recently been linked to three genetic pathways involved in dopamine/norepinephrine and serotonin neurotransmission, and neuritic outgrowth. In this study, we aimed to assess the association between ADHD symptoms, volume of the basal ganglia, and the three proposed genetic pathways in a pediatric general population sample. By doing so, we aimed to investigate the generalizability of earlier findings in a clinical population to the general population and gain knowledge regarding the neurobiology of ADHD symptomatology on a continuum.

Methods

This study is embedded within the Generation R Study and includes 1,871 children with data on ADHD symptoms and genetic data, and 344 children with additional neuroimaging data. We studied the relation between ADHD symptoms and basal ganglia volume. Next, we investigated the association between the three proposed genetic pathways (dopamine/norepinephrine, serotonin, neuritic outgrowth) and symptoms of ADHD, and the relation between the genetic pathways and basal ganglia volume.

Results

More inattention and hyperactivity/impulsivity symptoms were associated with a smaller volume of the putamen. The genetic pathways were not related to either ADHD symptoms or basal ganglia volume.

Conclusions

Our large population-based study supports a role of putamen volume in the neurobiology of ADHD problems. We found no evidence for a role of the dopamine/norepinephrine, serotonin and neuritic outgrowth genetic pathways in ADHD symptom severity.

INTRODUCTION

Attention-deficit/hyperactivity disorder (ADHD) is characterized by persistent inattention and/or hyperactivity and impulsivity problems that are thought to arise as a consequence of genetic risk factors, altered brain development, and environmental influences. The disorder has a complex and polygenic character, implying that many genes each of very small individual effect are involved. The clinical presentation of ADHD is highly heterogeneous, as not all children exhibit exactly the same set of problems and the same degree of severity. Although ADHD is one of the most common neurodevelopmental disorders with a worldwide prevalence of about 3-5% (Polanczyk, Willcutt, Salum, Kieling, & Rohde, 2014), little is known about its underlying neurobiology. In the current study, we aimed to assess the neurobiology of ADHD symptoms in a large population-based sample of children, by applying recently developed methods and using both genetic and brain imaging data.

Recent genetic studies have shown that genetic factors play a considerable role in the development of ADHD, with heritability estimates around 70% (Faraone et al., 2005; Nikolas & Burt, 2010; Posthuma & Polderman, 2013). In spite of the high heritability of ADHD, the identification of genes that are associated with the disorder has proven to be difficult. Genome-wide association studies (GWAS) that have been performed in the last years have not been successful (Neale et al., 2010) likely because the small effects of single genes require very large sample sizes to reach genome wide significance. To overcome the problems associated with this polygenic character of ADHD, new approaches have been sought, including gene-set analyses (Lips et al., 2012; Wang, Li, & Bucan, 2007). In gene-set analyses, single genes are combined in gene-sets that are jointly tested for association with the phenotype of interest. Compared to testing multiple separate genes or Single Nucleotide Polymorphisms (SNPs), gene-set analyses are generally more powerful, as they suffer less from multiple testing. In a recent study of Bralten et al. (2013) candidate genetic pathways, as represented by gene-sets, were tested in a large clinical ADHD sample (n=930) of children between 5 and 17 years of age. The authors selected three gene-sets based on their suspected relation with ADHD, namely the dopamine/norepinephrine pathway, the serotonin pathway and a pathway consisting of genes involved in neuritic outgrowth. Aberrant dopaminergic, noradrenergic and serotonergic neurotransmission has been frequently discussed as a potential causal pathway in ADHD (Caylak, 2012; Cortese, 2012; Faraone, Bonvicini, & Scassellati, 2014), and genes involved in neuritic outgrowth constituted the top results of ADHD GWAS studies (Poelmans, Pauls, Buitelaar, & Franke, 2011). In the study of Bralten et al. (2013) the three selected gene-sets were first tested against DSM-IV symptom count and, in a later step, against a continuous measure of ADHD symptom severity as measured using the Conners Parent and Teacher Rating Scales. The authors found the combination of the three genetic pathways, as well as each of the separate pathways, to be associated with DSM-IV symptom count of hyperactivity/impulsivity symptoms, but not with the count of Inattention symptoms. Analysis of symptom severity as rated with the Conners Parents Rating Scale validated this result (Bralten et al., 2013). As their sample included only subjects with a diagnosis of ADHD, they could only draw conclusions in the context of the association with symptom severity in a clinical sample. In order to test whether these results generalize to the full range symptom severity found in the general population, validation in a population-based sample is needed.

Identification of gene-sets with the severity of symptoms in ADHD suggests the involvement of specific biological pathways. However, in order to understand how these gene-sets may influence

ADHD symptom severity, we need insight into the functional consequences of genetic variation in the gene-sets. One route to investigate this is the use of intermediate phenotypes. Based on the criteria that define suitable intermediate phenotypes (Gottesman & Gould, 2003), brain morphology can be regarded a suitable intermediate phenotype in the association between genetics and ADHD. Studies investigating subcortical brain morphology in relation to ADHD symptoms have reported the most pronounced structural abnormalities in the basal ganglia (putamen, pallidum and caudate). A reduction of volume of these three structures has been consistently found in patients with ADHD (Frodl & Skokauskas, 2012; Nakao, Radua, Rubia, & Mataix-Cols, 2011; Shaw et al., 2014). As part of the cortico-striatal circuitry, the basal ganglia have been found to be modulated by different neurotransmitter systems, including the dopaminergic, serotonergic and noradrenergic systems (Carli & Invernizzi, 2014; Di Matteo et al., 2008) and reduced striatal dopamine availability has been previously reported in ADHD (Volkow et al., 2007).

The aim of our study was three-fold. First, in order to ascertain that our population-based sample holds a good representation of attention-deficit/hyperactivity problems (along a continuum) and to gain more insight in the neurobiology of these problems, we aimed to confirm the relation between volume of the basal ganglia structures and symptoms of ADHD as has been found in previous clinical samples. Second, we aimed to assess the association between the three genetic pathways as proposed by Bralten et al. (2013) and symptoms of ADHD, to test whether the earlier findings can be generalized to the general population. Finally, we tested the relation between the proposed genetic pathways and volume of the basal ganglia. We hypothesized that volume of the basal ganglia structures would be associated with symptoms of inattention and hyperactivity/impulsivity. Furthermore, in line with the study of Bralten et al. (2013), we expected that the three candidate genetic pathways would show an association with symptoms of hyperactivity/impulsivity. Lastly, we expected the dopamine/norepinephrine and serotonin pathways to also be associated with volume of the basal ganglia.

MATERIALS AND METHODS

Participants

This study is embedded within the Generation R Study, a population-based cohort, investigating children's health, growth and development from fetal life onwards in Rotterdam, the Netherlands. An overview of the Generation R Study design and population is described elsewhere (Jaddoe et al., 2012; Tiemeier et al., 2012). Around 8 years of age, the parents of 7,662 children were asked to fill out a Dutch version of the Conners Parent Rating Scale - Revised: Short Form (CPRS-R:S) (Conners, Sitarenios, Parker, & Epstein, 1998). Of this group, data of the CPRS-R:S Inattention and Hyperactivity/Impulsivity scales was available in 4,627 children. Next, we selected children (one child per family) that had GWAS data available and were European Caucasian, resulting in a total of 1,871 children for the genetic analyses. At age 6 years, a brain Magnetic Resonance Imaging (MRI) study began within a subsample of the Generation R Study. Between September 2009 and July 2013, a total of 1,070 children were scanned (White et al., 2013). Of the 1,871 Caucasian children with GWAS and CPRS-R:S data, 388 children also received an MRI scan, with 344 of these children having good quality structural imaging data.

The study was approved by the Medical Ethics Committee (METC) of the Erasmus Medical Center

and the Central Committee of Research involving Human Subjects (CCMO). Written informed consent was obtained from the parents of all participants.

Inattention and Hyperactivity/Impulsivity Symptoms

Data regarding symptoms of inattention and hyperactivity/impulsivity were obtained by administering the Dutch version of the CPRS-R:S (Conners et al., 1998). The CPRS-R:S is the short form of the original CPRS-R questionnaire, consisting of 27 items that are to be filled out by the parent or primary caregiver of the child, reporting on problem behavior (oppositional, cognitive problems/inattention, hyperactivity) of their child in the preceding month. All questions were scored on a 4-point scale, ranging from 0 as 'not true at all' up to 3 as 'very much true'. In all analyses, the raw sumscores of the Inattention and Hyperactivity/Impulsivity scales of the CPRS-R:S were used. The scores were right skewed, because (as can be expected) many children in our population-based sample had little attention and hyperactivity/impulsivity problems. Therefore, we additionally ran analyses on Blom transformed (inverse normal transformation) CPRS-R:S scores. For descriptive purposes, the percentage of children that scored above the cut off indicating clinically significant problems ($t > 65$) according to the manual (Conners, 1997) is provided.

Brain Imaging

MR images were acquired using a GE Discovery MR750 3.0 Tesla scanner (GE Healthcare Worldwide, Milwaukee, WI, USA) with an 8-channel head coil. The high-resolution T1-weighted image was collected using an inversion recovery fast spoiled gradient recalled (IR-FSPGR) sequence with the following parameters: TR = 10.3 ms, TE = 4.2 ms, TI = 350 ms, NEX = 1, flip angle = 16°, readout bandwidth= 20.8 kHz, matrix 256 x 256, imaging acceleration factor of 2, and an isotropic resolution of 0.9x0.9x0.9 mm^3. Before scanning took place, children were familiarized with the scanning environment during a mock scanning session. All procedures have been described in detail elsewhere (White et al., 2013). Cortical reconstruction and volumetric segmentation was performed with the FreeSurfer image analysis suite (http://surfer.nmr.mgh.harvard.edu/), version 5.1. Image quality assurance was performed in 2 steps; first as a visual inspection of the image quality of the T1 sequence prior to preprocessing the data and second as a visual inspection of the segmentation quality after the images were processed through the FreeSurfer pipeline. The (technical) details of these procedures have been described previously (Mous et al., 2014).

Subcortical volume regions of interest (ROIs) were chosen based on their involvement in ADHD in previous literature (Bush, Valera, & Seidman, 2005; Frodl & Skokauskas, 2012; Nakao et al., 2011) and consisted of structures of the basal ganglia (putamen, pallidum, caudate). Because we did not expect laterality differences, volumes of the left and right hemispheres were summed and analyzed jointly.

Candidate Genetic Pathways

DNA was collected from cord blood samples at birth or from blood samples at 6 years of age. Genotyping was performed using Illumina Infinium II HumanHap 610 or 660W Quad Arrays following standard manufacturer's protocols and quality control was performed. Exclusion criteria for samples were: duplicates, gender mismatch, relatedness, excess of heterozygosity, and call rate <97.5%. Exclusion criteria for SNPs were: MAF <1%, missingness >0.05, call rate <98%, and HWE $p \leq 0^{-6}$ (Medina-

Gomez et al., 2012). All SNPs that passed quality control were mapped to genes based on the National Center for Biotechnology Information (NCBI) human assembly build 36. The genetic pathways and their associated genes were chosen similar to the study of Bralten et al. (2013). In this previous study three candidate pathways for ADHD were selected (dopamine/norepinephrine, serotonin and neuritic outgrowth), of which gene selection was based on a genetic database (for the first two pathways) or from the literature (for the third pathway). The dopamine/norepinephrine pathway consisted of 82 genes, the serotonin pathway of 32 genes and the neuritic outgrowth pathway of 45 genes. As the dopamine/norepinephrine pathway and the serotonin pathway shared 13 genes, the total gene-set contained 146 unique genes. Of this number, 20 genes were not captured by the array that was used in our study and were therefore not included in the analyses, resulting in a final total gene-set of 126 unique genes. The final number of genes in the separate pathways was 67 (1208 SNPs) for the dopamine/norepinephrine pathway, 28 genes (290 SNPs) for the serotonin pathway and 43 genes (6006 SNPs) for the neuritic outgrowth pathway. For the individual genes within these pathways, see Supplementary Tables S3.1 and S3.2.

Additional Measures

In Table 3.1, participant characteristics are presented for both the total sample with genetic data, as well as the imaging subsample. The selection of children from European Caucasian descent was performed based on genotype data. To define child ethnicity for descriptive purposes, the ethnicity categorization of Statistics Netherlands (Statistics Netherlands, 2004a) was used. Children with both parents born in the Netherlands were considered Dutch and children were classified as non-Dutch (further categorized as 'other Western' or 'other non-Western') if at least one parent was born outside the Netherlands. Information regarding the date of birth and gender was obtained from midwives or hospital registries. The use of psychostimulant medication was recorded during the visit for the MRI scan. As ADHD symptoms have been shown to be comorbid with autistic traits, analyses were adjusted for autistic traits, as measured with the Dutch version of the Social Responsiveness Scale (SRS) questionnaire (Constantino & Gruber, 2002). Maternal education was defined as highest education completed, according to the definition of Statistics Netherlands (Statistics Netherlands, 2004b) and household income was defined by the total net monthly income of the household. Information on maternal alcohol use and smoking during pregnancy was obtained using questionnaires in each trimester of pregnancy. Missing values of these potential confounding environmental or risk factors were imputed.

TABLE 3.1. **PARTICIPANT CHARACTERISTICS**

	TOTAL SAMPLE	IMAGING SUBSAMPLE
CHILD CHARACTERISTICS	**n=1871**	**n=344**
Gender, % boys	50.9	50.3
Ethnicity, %[a]		
• Dutch	89.8	94.5
• Other Western	8.4	4.1
• Non-Western	1.8	1.4
Age brain imaging, years[b]	-	8.08 (0.97)
Age Conners' (CPRS-R:S) assessment, years	8.15 (0.22)	8.19 (0.25)
Conners' (CPRS-R:S) Inattention		
• Score	3.10 (3.59)	3.56 (3.92)
• Range, min - max	0 - 18	0 - 18
• T-score moderately atypical (65<T≤70), %	2.8	2.9
• T-score markedly atypical (T>70), %	5.2	7.3
Conners' (CPRS-R:S) Hyperactivity/Impulsivity		
• Score	2.09 (2.71)	2.70 (3.33)
• Range, min – max	0 - 18	0 - 18
• T-score moderately atypical (65<T≤70), %	3.0	4.1
• T-score markedly atypical (T>70), %	2.7	6.1
Psychostimulant use, % yes[b]	-	3.2
Social Responsiveness Scale (SRS), score	0.19 (0.21)	0.23 (0.25)
MATERNAL CHARACTERISTICS		
Education level, %		
• High	75.5	72.1
• Medium	19.7	22.4
• Low	4.8	5.5
Alcohol use during pregnancy, %		
• Never	24.5	25.6
• Until pregnancy was known	14.4	14.8
• Continued occassionally during pregnancy	47.3	44.5
• Continued frequently during pregnancy[c]	13.8	15.1
Smoking during pregnancy, %		
• Never	80.4	80.8
• Until pregnancy was known	8.1	6.4
• Continued during pregnancy	11.5	12.8
Household income, %		
• >2000 euro	93.2	89.8
• 1200-2000 euro	5.2	7.3
• <1200 euro	1.6	2.9

NOTE. Values given as mean and standard deviation, unless otherwise indicated. [a] Categories include only ethnicities that are regarded Caucasian. [b] Data only collected in imaging subsample. [c] Frequent continued use was defined as one drink or more per week during at least 2 trimesters of pregnancy.

Statistical Analyses

The statistical analyses were carried out in three steps, as explained in the sections below and Figure 3.1. First, we studied the association between volume of the basal ganglia structures and symptoms of inattention and hyperactivity/impulsivity, by performing linear regression analyses (1). Second, the association between the candidate genetic pathways and symptoms of inattention and hyperactivity/impulsivity was studied, by performing gene-set association analyses (2). Lastly, we again performed gene-set analyses, assessing the association between the genetic pathways and volume of the basal ganglia (3).

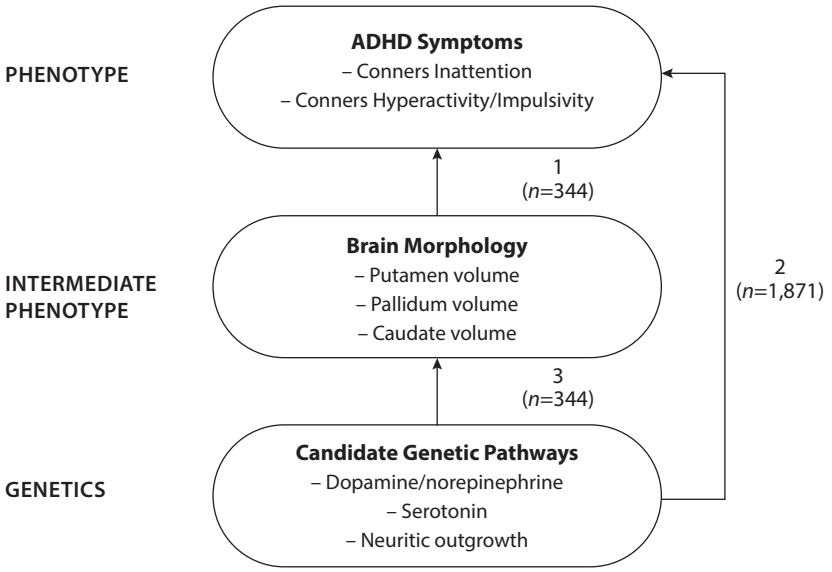

FIGURE 3.1. Overview analyses steps

Imaging Association Analyses

The imaging analyses were performed using ROI data generated by the FreeSurfer analysis stream. We performed linear regression analyses in SPSS (version 21), testing the association of both the Inattention and Hyperactivity/Impulsivity scales of the CPRS-R:S with volume of the three selected basal ganglia structures (putamen, pallidum, caudate). To correct for multiple testing, Bonferroni correction was applied to the association analyses of the three separate structures. Because of the strong correlations between the volumes of the three regions (Pearson correlation ranging between r=0.5 and r=0.7), we first calculated the effective number of tests (M_{eff}) (Galwey, 2009) and adjusted the Bonferroni correction accordingly to account for this lack of independence. The calculation yielded an effective number of 2.54 tests, which resulted in a corrected significance threshold of $\alpha = 0.05 / 2.54 = 0.020$. All imaging association analyses were corrected for age, gender and total brain volume. Other covariates were added to the regression analyses as potential confounders only if they changed the effect estimate (B) with 5% or more. These included ethnicity, the SRS score of autistic traits and psychostimulant use.

Genetic Association Analyses

The gene-set analyses were performed using the software package Joint Association of Genetic Variants (JAG) (Lips et al., 2012). First, a self-contained test was performed, assessing the joint effect of SNPs within all unique genes of the three pathways together, following the analysis of Bralten et al. (2013). Subsequently, the effect of the three single pathways was tested. Empirical p-values were computed by performing 100,000 permutations of the phenotype. Since we used SNP p-values rather than raw data, we used the genotype data of the European ancestry samples from the 1000 Genomes project (Genomes Project et al., 2012) with a simulated binary phenotype as a reference dataset under the null hypothesis, removing all SNPs with a minor allele count smaller than four. For our dataset we selected only SNPs present in the 1000 Genomes raw data for optimal resemblance with the reference dataset. To account for multiple testing, Bonferroni correction was applied to the association analyses of the three separate pathways. The corrected significance threshold was set at $\alpha = 0.05 / 3 = 0.017$. For any pathways found to be significant after correction for multiple testing a competitive test was performed, which assesses whether the observed association is stronger than expected for random sets of genes of the same size. Only pathways with a competitive p-value smaller than 0.05 were considered to be significant and truly informative. All genetic association analyses were corrected for age and gender and four principal components were included to correct for population stratification.

First, we performed the gene-set analyses testing the association with the Inattention and Hyperactivity/Impulsivity scales of the CPRS-R:S. Similarly, we performed gene-set analyses testing the association with volume of the three basal ganglia structures. For each gene-set we also inspected gene-based p-values, to assess evidence of heterogeneity of association across genes within a set. Gene-based results are provided as supplementary tables.

RESULTS

Volume Basal Ganglia & Inattention and Hyperactivity/Impulsivity

The results of the imaging analyses, testing the association between volume of the three basal ganglia structures and the CPRS-R:S Inattention and Hyperactivity/Impulsivity scores, are shown in Table 3.2. After correction for potential confounding factors and multiple testing, we found volume of the putamen to be significantly associated with both the CPRS-R:S Inattention (β=-0.15, p=0.019) and Hyperactivity/Impulsivity (β=-0.15, p=0.018) scores. Children with more inattention or hyperactivity/impulsivity problems had smaller volumes of the putamen. Volume of the other basal ganglia structures (pallidum and caudate) was not significantly associated with either the Inattention or Hyperactivity/Impulsivity scores. Analyses using Blom transformed CPRS-R:S scores yielded similar results.

Genetic Pathways & Inattention and Hyperactivity/Impulsivity

Table 3.3 shows the results of the gene-set analyses testing the association between the three genetic pathways and the CPRS-R:S Inattention and Hyperactivity/Impulsivity scores. The joint effect of all genes of the three pathways showed no significant association with either the Inattention or the Hyperactivity/Impulsivity scores of the CPRS-R:S in the self-contained test. The three separate pathways (dopamine/norepinephrine, serotonin and neuritic outgrowth) were also not significantly asso-

ciated with either of the CPRS-R:S scores. Analyses using Blom transformed CPRS-R:S scores yielded similar results. Consequently, no competitive tests were performed. Gene-based results are shown in Supplementary Table S3.1 and Supplementary Figure S3.1, showing that the separate genes within the gene-sets were not significantly related to the CPRS-R:S Inattention and Hyperactivity/Impulsivity scores after correction for multiple testing.

Genetic Pathways and Volume Basal Ganglia

The results of the gene-set analyses testing the association between the genetic pathways and volume of the basal ganglia structures are shown in Table 3.4. Both the joint effect of all three pathways, as well as the three separate pathways (dopamine/norepinephrine, serotonin and neuritic outgrowth) did not show a significant association with any of the basal ganglia structures in the self-contained test. Consequently, no competitive tests were performed. Gene-based results are shown in Supplementary Table S3.2 and Supplementary Figure S3.1, showing that the separate genes within the gene-sets were not significantly related to volume of the putamen, pallidum or caudate after correction for multiple testing.

TABLE 3.2. **ASSOCIATION OF VOLUMES (CM^3) WITH CONNERS' SCORES**

	MODEL I			MODEL II			MODEL III		
	B (95% CI)	**BETA**	**P-VALUE ($\alpha = 0.020$)**	**B (95% CI)**	**BETA**	**P-VALUE ($\alpha = 0.020$)**	**B (95% CI)**	**BETA**	**P-VALUE ($\alpha = 0.020$)**
INATTENTION SCORE									
Putamen	-0.575 (-0.891;-0.258)	-0.20	**<0.001**	-0.483 (-0.855;-0.111)	-0.17	**0.011**	-0.427 (-0.784;-0.069)	-0.15	**0.019**
Pallidum	-0.987 (-2.086;0.112)	-0.10	0.078	-0.306 (-1.587;0.976)	-0.03	0.639	-0.414 (-1.639;0.811)	-0.04	0.507
Caudate	-0.338 (-0.709;0.034)	-0.10	0.075	-0.051 (-0.521;0.418)	-0.02	0.830	-0.064 (-0.515;0.386)	-0.02	0.778
HYPERACTIVITY/IMPULSIVITY SCORE									
Putamen	-0.474 (-0.745;-0.203)	-0.19	**0.001**	-0.404 (-0.723;-0.085)	-0.16	**0.013**	-0.362 (-0.661;-0.063)	-0.15	**0.018**
Pallidum	-0.423 (-1.366;0.519)	-0.05	0.378	0.262 (-0.835;1.359)	0.03	0.639	-0.095 (-0.930;1.120)	0.01	0.855
Caudate	-0.308 (-0.626;0.009)	-0.10	0.057	-0.100 (-0.503;0.302)	-0.03	0.624	-0.121 (-0.497;0.255)	-0.04	0.528

NOTE. n=344. Model I adjusted for age and gender. Model II = model I + total brain volume. Model III = model II + ethnicity, SRS score and psychostimulant use. Significant results are **bold**.

TABLE 3.3. **ASSOCIATION OF GENETIC PATHWAYS WITH CONNERS SCORES**

				CPRS-R:S INATTENTION SCORE		CPRS-R:S HYPERACTIVITY/ IMPULSIVITY SCORE	
PATHWAY	NUMBER OF GENES IN ORIGINAL SET	NUMBER OF GENES PRESENT IN GWAS DATA	NUMBER OF SNPs PRESENT IN GWAS DATA	SELF-CONTAINED P-VALUE ($\alpha = 0.017$)	COMPETITIVE P-VALUE ($\alpha = 0.05$)	SELF-CONTAINED P-VALUE ($\alpha = 0.017$)	COMPETITIVE P-VALUE ($\alpha = 0.05$)
All genes	146	126	7388	0.694[a]	NA	0.431[a]	NA
Dopamine/Norepinephrine	82	67	1208	0.824	NA	0.568	NA
Serotonin	32	28	290	0.560	NA	0.584	NA
Neuritic outgrowth	45	43	6006	0.591	NA	0.368	NA

NOTE. n=1,871. [a] Significance level α=0.05. NA=not applicable.

TABLE 3.4. **ASSOCIATION OF GENETIC PATHWAYS WITH VOLUME OF BASAL GANGLIA STRUCTURES**

				VOLUME PUTAMEN		VOLUME PALLIDUM		VOLUME CAUDATE	
PATHWAY	NUMBER OF GENES IN ORIGINAL SET	NUMBER OF GENES PRESENT IN GWAS DATA	NUMBER OF SNPs PRESENT IN GWAS DATA	SELF-CONTAINED P-VALUE ($\alpha = 0.017$)	COMPETITIVE P-VALUE ($\alpha = 0.05$)	SELF-CONTAINED P-VALUE ($\alpha = 0.017$)	COMPETITIVE P-VALUE ($\alpha = 0.05$)	SELF-CONTAINED P-VALUE ($\alpha = 0.017$)	COMPETITIVE P-VALUE ($\alpha = 0.05$)
All genes	146	126	7391	0.882[a]	NA	0.859[a]	NA	0.305[a]	NA
Dopamine / Norepinephrine	82	67	1208	0.862	NA	0.990	NA	0.669	NA
Serotonin	32	28	290	0.890	NA	0.700	NA	0.287	NA
Neuritic outgrowth	45	43	6006	0.773	NA	0.618	NA	0.241	NA

NOTE. n=344. [a] Significance level α=0.05. NA=not applicable.

DISCUSSION

In the current large population-based sample of children we found a smaller volume of the putamen to be associated with more inattention and hyperactivity/impulsivity symptoms. We did not find support for a role of the previously identified dopamine/norepinephrine, serotonin and neuritic outgrowth genetic pathways (Bralten et al., 2013) in ADHD symptom severity or basal ganglia volume in our population-based sample.

The notion that child psychopathology, such as ADHD, does not necessarily fall within diagnostic categories with clearly defined boundaries, but that symptoms may be better described within a dimensional framework (covering the entire spectrum of problems) has gained support over the last years. Numerous studies demonstrate that such a dimensional approach can further contribute to a better etiological understanding of child psychopathology (Hudziak, Achenbach, Althoff, & Pine, 2007; Lubke, Hudziak, Derks, van Bijsterveldt, & Boomsma, 2009; Polderman et al., 2007). Consequently, dimensional approaches becoming increasingly popular and are being evaluated as a part of the National Institutes

of Mental Health's Research Domain Criteria (Insel et al., 2010). Despite these developments, the majority of the current (neurobiological) studies are still performed in a case-control design or solely within a clinical sample. As discussed by Bralten et al. (2013), the association of the dopamine/norepinephrine, serotonin and neuritic outgrowth genetic pathways that they found with symptoms of ADHD should be replicated in a population-based sample. Replication in a population-based sample would support the notion of dimensionality of (the neurobiology of) attention and hyperactivity problems. This would make it possible to extend previously drawn conclusions based on clinical samples to the full range of problems in the general population.

An important strength of our study is the use of gene-set analyses, instead of testing single genes or SNPs. Gene-set analysis takes the polygenic character of ADHD into account, increases power by reducing multiple testing (as compared to testing multiple single genes or SNPs), and enhances interpretability of findings. Using gene-sets of which all genes function within a specific biological pathway provides a more direct route to knowledge about the underlying neurobiology of the disorder, whereas interpreting the effect of a single gene or SNP may be more difficult. Second, we have tested an intermediate phenotype in the association between genetics and ADHD problems. By doing so, we not only wanted to ascertain that our sample holds a good representation of ADHD symptoms, but we also aimed to acquire more knowledge with regard to the neurobiology underlying ADHD symptoms. Another strength is the population-based nature of our study. By using a population-based sample and a continuous score for ADHD symptoms, our study provides greater generalizability with the general population compared to a study sample recruited from a clinical setting. Furthermore, we were able to correct the imaging analyses for the use of psychostimulant medication. As psychostimulant use may normalize brain structure in children with ADHD (Frodl & Skokauskas, 2012; Nakao et al., 2011; Rubia, Alegria, & Brinson, 2014) and reduces inattention and hyperactivity symptoms, this is a very important potentially confounding factor to take into account.

As hypothesized, our analyses showed an association between of the basal ganglia and inattention and hyperactivity/impulsivity symptoms in our population-based sample. Specifically, we found volume of the putamen to be associated. In line with studies in clinical ADHD, children with more inattention and hyperactivity/impulsivity problems had smaller volumes in this structure. The other basal ganglia structures, the pallidum and caudate, were not related to symptoms of ADHD. Although most research regarding the functions of the basal ganglia has focused on its known role in motor behaviour, other studies have also shown the basal ganglia (and specifically the putamen) to be involved in (somato)sensory, affective, working memory and other higher order executive functioning processes (see (Arsalidou, Duerden, & Taylor, 2013) for an extensive meta-analysis), (cognitive) functions that are typically impaired in ADHD.

Despite the large sample size of our study (n=1,871), we were unable to replicate the association between the three proposed candidate genetic pathways and symptoms of hyperactivity and impulsivity. This implies that, if any effect is present at all, it is most likely to be a very small effect. Although the variation (range) in scores on the CPRS-R:S is larger in a population-based sample like ours (as it covers the entire spectrum of problems, from no problems to clinically significant problems), the scores were right skewed as many children in the general population do not have many problems. This is a limitation of our study and could potentially have reduced the power. When repeating the analyses on Blom transformed (inverse normal transformation) CPRS-R:S scores results remained similar, suggesting that

the distribution of scores in our sample is unlikely to explain the observed lack of association. Although our study was performed in a general population sample, which is less severely affected compared to a clinical population (such as the sample of Bralten et al., 2013), our sample did include children that had clinically elevated scores (8% for inattention and 6% for hyperactivity/impulsivity symptom scores). Furthermore, the short form of the CPRS-R was collected in the current study, instead of the full CPRS-R. This short form, which contains less questions, might have negatively affected the variability in scores. Although our sample was fairly large, we might need an even larger sample to find significant effects. Another potential explanation for the difference in findings might be that the study of Bralten et al. (2013), as the first study to report this association, may have overestimated the true effect (i.e. a winner's curse) and that the true effect, if present, is much smaller and necessitates larger sample sizes than present in our study.

Since volume of the putamen was found to be associated with symptoms of ADHD, this feature of brain morphology may indeed serve as a suitable intermediate phenotype in studying the biological pathway between the genetics and ADHD symptoms. Following this argumentation, one would expect dopamine/norepinephrine, serotonin or neuritic outgrowth pathways to be related to putamen volume. Contrary to this expectation, we did not find volume of the putamen (or any of the other two basal ganglia structures) to be related to any of the three genetic pathways. However, since our sample of Caucasian children with genetic and brain imaging data available was rather small (n=344), the association between genetic pathways related to ADHD and volume of the putamen should be further studied in larger samples.

To conclude, in a large population-based sample of children we found a smaller volume of the putamen to be associated with higher levels of inattention and hyperactivity/impulsivity problems. We were unable to replicate the previously found (Bralten et al., 2013) association of candidate genetic pathways involved in dopamine/norepinephrine and serotonin neurotransmission and neuritic outgrowth with hyperactivity/impulsivity symptom severity. Furthermore, we did not find the proposed genetic pathways to be associated with volume of the putamen. Given the positive association between volume of the putamen and ADHD symptom severity, this suggests that the tested candidate gene-sets are not or at most only weakly associated with ADHD symptoms. Taken together, our findings (i) support a role of volume of the putamen in the neurobiology of ADHD symptoms, and (ii) do not support a role of the dopamine/norepinephrine, serotonin and neuritic outgrowth genetic pathways in ADHD symptom severity in a population-based sample.

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SUPPLEMENT

TABLE S3.1. **GENE-BASED ASSOCIATIONS WITH CONNERS INATTENTION AND HYPERACTIVITY/IMPULSIVITY SYMPTOMS**

ENTREZ GENE	PART OF PATHWAY			NUMBER OF SNPs	ASSOCIATION P-VALUE ($\alpha = 0.0004$)[a]	
	DOPAMINE / NOREPINEPHRINE	SEROTONIN	NEURITIC OUTGROWTH		INATTENTION	HYPERACTIVITY / IMPULSIVITY
ADAMTS17			x	120	0.1148	0.1302
ADCY1	x			30	0.4807	0.4907
ADCY10	x			31	0.8910	0.7254
ADCY2	x			113	0.9341	0.4315
ADCY3	x			19	0.4322	0.2856
ADCY4	x			4	0.0521	0.2066
ADCY5	x			26	**0.0274***	0.2525
ADCY6	x			5	0.9975	0.3900
ADCY7	x			6	0.6499	0.2784
ADCY8	x			75	0.7034	0.4366
ADCY9	x			58	0.7299	0.1988
ADRA1A	x			53	0.7627	0.7986
ADRA1B	x			15	0.3562	0.4655
ADRA1D	x			6	0.5643	0.7802
ADRA2A	x			1	0.9363	0.7918
ADRA2B	x			3	0.4595	0.1630
ASTN2			x	302	0.7067	0.7308
ATP2C2			x	84	0.7354	0.4944
BMPR1B			x	84	0.6045	0.6121
CALY	x			2	0.5765	0.6892
CCSER1			x	70	0.6203	0.5960
CDH13			x	667	0.6479	0.3118
CDH23			x	159	0.0754	0.5678
COMT	x			12	0.2460	**0.0233***
CREB5			x	185	0.5261	0.5098
CSMD2			x	200	0.7229	0.6459
CTNNA2			x	363	0.7415	0.8040
DDC	x	x		33	0.6504	0.3415
DNM1			x	11	0.3960	0.4152
DRD1	x			3	0.8050	0.1985
DRD2	x			17	0.9222	0.9602
DRD3	x			13	0.9580	0.2361
DYNC2H1			x	65	**0.0125***	**0.0238***
EMP2			x	20	0.3520	0.4669
FHIT			x	629	0.8259	0.2939
FLNC			x	2	**0.0013*****	0.3895
GCH1	x	x		3	0.4189	0.7727
GPC6			x	253	0.5291	0.1563
HK1			x	44	0.8988	0.7084

TABLE S3.1. **GENE-BASED ASSOCIATIONS WITH CONNERS INATTENTION AND HYPERACTIVITY/IMPULSIVITY SYMPTOMS** (CONT.)

ENTREZ GENE	PART OF PATHWAY			NUMBER OF SNPs	ASSOCIATION P-VALUE ($\alpha = 0.0004$)[a]	
	DOPAMINE / NOREPINEPHRINE	SEROTONIN	NEURITIC OUTGROWTH		INATTENTION	HYPERACTIVITY / IMPULSIVITY
HKDC1			x	22	0.7456	0.9129
HTR1E		x		10	0.3622	0.8554
HTR2A		x		32	0.7906	0.4978
HTR2B		x		3	0.7136	0.7597
HTR2C		x		12	0.0705	**0.0136***
HTR3A		x		8	**0.0468***	0.6165
HTR3B		x		5	0.4341	0.6068
HTR3C		x		2	0.3071	0.0634
HTR3D		x		5	0.5000	0.7829
HTR3E		x		2	0.5919	0.7536
HTR4		x		35	0.3770	0.9063
HTR5A		x		2	0.6961	0.8584
HTR6		x		3	0.2083	0.9151
HTR7		x		16	0.7888	0.4547
IL4I1	x	x		11	0.3055	0.4724
ITGA11			x	63	0.4225	0.4790
KCNIP4			x	300	0.5896	0.6108
LRP1B			x	484	0.1908	0.1278
MAN2A2			x	4	0.0619	0.6341
MAOA	x	x		5	0.4147	0.5147
MAOB	x	x		4	0.3873	0.1635
MAP1B			x	32	0.9695	0.8280
MBOAT1			x	41	0.7872	0.3381
MEIS2			x	61	0.6563	0.6914
MMP24			x	7	0.4264	0.0787
MOBP			x	16	0.0529	0.2409
MYT1L			x	88	0.5573	0.7794
NCKAP5			x	210	0.6957	0.3237
NCS1	x			20	0.1838	0.5100
NEDD4L			x	111	0.1578	0.7300
NOS1			x	39	0.7892	0.6318
NRXN1			x	293	0.3678	0.8293
NUCB1			x	5	0.7642	0.8984
NXPH1			x	105	0.2749	0.1416
PCBD1	x	x		1	1	0.8824
PPM1H			x	111	0.8624	0.1883
PPM1L	x			38	0.3953	0.2148
PPP1CB	x			6	0.3127	0.2663

TABLE S3.1. **GENE-BASED ASSOCIATIONS WITH CONNERS INATTENTION AND HYPERACTIVITY/IMPULSIVITY SYMPTOMS** (CONT.)

ENTREZ GENE	PART OF PATHWAY			NUMBER OF SNPs	ASSOCIATION P-VALUE ($\alpha = 0.0004$)[a]	
	DOPAMINE / NOREPINEPHRINE	SEROTONIN	NEURITIC OUTGROWTH		INATTENTION	HYPERACTIVITY / IMPULSIVITY
PPP1CC	x			4	0.5224	0.1282
PPP1R10	x			2	0.3476	0.8588
PPP1R11	x			1	**0.0229***	0.1697
PPP1R12A	x			4	**0.0416***	**0.0119***
PPP1R14B	x			1	0.1593	0.9649
PPP1R14C	x			37	0.3819	0.6060
PPP1R3A	x			3	0.4769	0.7014
PPP1R7	x			7	0.9440	0.5164
PPP2CB	x			3	0.8681	0.9179
PPP2R1A	x			18	0.6778	0.5575
PPP2R1B	x			2	0.6067	0.9891
PPP2R2A	x			14	0.2574	0.1154
PPP2R2B	x			98	0.1419	0.8935
PPP2R2C	x			64	0.9728	0.9510
PPP2R3A	x			12	0.1271	0.5845
PPP2R3B	x			1	0.3931	0.7103
PPP2R4	x			6	0.8511	0.8616
PPP2R5A	x			6	0.3278	**0.0023*****
PPP2R5B	x			1	0.2189	0.3447
PPP2R5C	x			18	0.0515	0.3412
PPP2R5D	x			3	0.5379	0.5192
PPP2R5E	x			18	0.4911	0.7779
PRKACA	x			1	0.8879	0.8290
PRKACB	x			17	0.0917	0.2336
PRKAG1	x			1	0.2723	0.3642
PRKAG2	x			112	0.8270	0.8425
PRKAR1A	x			4	0.3987	0.9430
PRKAR2A	x			3	0.0685	**0.0034*****
PRKAR2B	x			19	0.8034	0.6263
PTS	x	x		1	0.8916	0.7181
QDPR	x	x		9	0.5973	**0.0143***
RORA			x	304	**0.0366***	0.5439
SLC18A1	x	x		18	0.9082	0.4340
SLC18A2	x	x		19	0.4270	0.4968
SLC18A3	x	x		1	0.5204	0.5199
SLC6A2	x			32	0.2033	0.0839
SLC6A3	x			25	0.8920	0.7493
SLC6A4		x		10	0.7523	0.8301
SLCO3A1			x	112	0.5992	0.9079

TABLE S3.1. **GENE-BASED ASSOCIATIONS WITH CONNERS INATTENTION AND HYPERACTIVITY/IMPULSIVITY SYMPTOMS** (CONT.)

ENTREZ GENE	PART OF PATHWAY			NUMBER OF SNPs	ASSOCIATION P-VALUE (α = 0.0004)[a]	
	DOPAMINE / NOREPINEPHRINE	SEROTONIN	NEURITIC OUTGROWTH		INATTENTION	HYPERACTIVITY / IMPULSIVITY
SMOX	x	x		8	0.1353	0.9389
SPOCK3			x	78	0.7953	0.9181
SUPT3H			x	56	0.6624	0.5123
TH	x			2	0.1929	0.2080
TLL2			x	40	0.8928	**0.0413***
TPH1		x		4	0.9921	0.5941
TPH2		x		28	0.2449	0.3617
UGT1A9			x	46	0.7531	0.2653
UNC5B			x	22	0.2475	0.1750
ZNF423			x	98	0.2627	0.1828

NOTE. n=344. Number of genes dopamine/norepinephrine pathway=67, number of genes serotonin pathway=28, number of genes neuritic outgrowth pathway=43. The strongest found associations are indicated: *** p-value <0.005, ** p-value between 0.005 and 0.01, * p-value between 0.01 and 0.05. [a] Corrected threshold for significance was set at p=0.0004 (0.05/126 genes).

TABLE S3.2. **GENE-BASED ASSOCIATIONS WITH VOLUME OF PUTAMEN, PALLIDUM AND CAUDATE**

ENTREZ GENE	PART OF PATHWAY			NUMBER OF SNPs	ASSOCIATION P-VALUE ($\alpha = 0.0004$)[a]		
	DOPAMINE / NOREPI-NEPHRINE	SEROTONIN	NEURITIC OUTGROWTH		PUTAMEN	PALLIDUM	CAUDATE
ADAMTS17			x	120	0.7527	0.9705	0.9932
ADCY1	x			30	0.2610	0.6972	0.6214
ADCY10	x			31	0.8876	0.2578	0.9555
ADCY2	x			113	0.7302	0.7288	0.2772
ADCY3	x			19	0.0927	0.9469	0.4973
ADCY4	x			4	0.5244	0.9716	0.8412
ADCY5	x			26	0.7304	0.6387	0.7219
ADCY6	x			5	0.0561	0.8440	0.2918
ADCY7	x			6	0.4095	0.9863	0.0519
ADCY8	x			75	0.0578	0.9266	0.8864
ADCY9	x			58	0.8516	0.9562	0.2235
ADRA1A	x			53	0.6674	0.5859	0.5879
ADRA1B	x			15	0.6488	0.6796	0.4178
ADRA1D	x			6	0.3597	0.4581	0.8649
ADRA2A	x			1	0.2997	0.4340	0.4360
ADRA2B	x			3	**0.0026*****	0.0582	0.6088
ASTN2			x	302	0.5679	0.8492	0.1449
ATP2C2			x	84	0.4485	0.5448	0.3256
BMPR1B			x	84	0.4499	0.5923	0.0723
CALY	x			2	0.9736	0.4180	**0.0460***
CCSER1			x	70	0.8907	0.3750	0.8726
CDH13			x	667	0.5652	0.3382	0.6609
CDH23			x	159	0.9331	0.8142	0.3565
COMT	x			12	0.6975	0.7484	0.7744
CREB5			x	185	0.0930	0.5617	0.4423
CSMD2			x	200	0.4320	0.3907	0.9828
CTNNA2			x	363	0.6473	0.3346	0.0634
DDC	x	x		33	0.6703	0.3664	0.4045
DNM1			x	11	0.9357	0.2249	0.0972
DRD1	x			3	0.4638	0.3344	0.0503
DRD2	x			17	0.8889	0.7662	0.6874
DRD3	x			13	0.1315	0.9332	0.4769
DYNC2H1			x	65	0.8431	0.8476	**0.0129***
EMP2			x	20	0.2936	0.8758	**0.0212***
FHIT			x	629	0.5430	0.9803	0.2759
FLNC			x	2	0.1964	0.0896	0.5134
GCH1	x	x		3	0.6045	0.3050	0.8275
GPC6			x	253	0.7323	0.2227	0.9845
HK1			x	44	**0.0281***	0.4886	**0.0164***
HKDC1			x	22	0.5716	0.2957	0.7222

TABLE S3.2. **GENE-BASED ASSOCIATIONS WITH VOLUME OF PUTAMEN, PALLIDUM AND CAUDATE** (CONTINUED)

ENTREZ GENE	PART OF PATHWAY			NUMBER OF SNPs	ASSOCIATION P-VALUE ($\alpha = 0.0004$)[a]		
	DOPAMINE / NOREPI-NEPHRINE	SEROTONIN	NEURITIC OUTGROWTH		PUTAMEN	PALLIDUM	CAUDATE
HTR1E		x		10	0.1668	0.3891	0.4585
HTR2A		x		32	0.5732	0.7647	0.9265
HTR2B		x		3	0.4717	0.3181	0.2462
HTR2C		x		12	0.6465	0.2314	0.5875
HTR3A		x		8	0.1668	0.0856	0.3109
HTR3B		x		5	0.2712	0.6709	0.3649
HTR3C		x		2	0.7833	0.6194	0.1917
HTR3D		x		5	0.4439	0.8852	0.6472
HTR3E		x		2	0.9177	0.4972	0.5538
HTR4		x		35	0.4222	0.3161	0.4951
HTR5A		x		2	0.8998	0.4773	0.4761
HTR6		x		3	0.9064	0.8599	**0.0192***
HTR7		x		16	0.9886	0.9575	**0.0136***
IL4I1	x	x		11	0.7315	0.2124	**0.0262***
ITGA11			x	63	0.4992	0.8691	0.5759
KCNIP4			x	300	0.1575	**0.0364***	0.1593
LRP1B			x	484	0.8127	0.1121	0.3960
MAN2A2			x	4	0.4096	0.0970	0.8364
MAOA	x	x		5	0.1833	0.7965	0.5808
MAOB	x	x		4	0.8617	0.7132	0.4788
MAP1B			x	32	0.8900	0.7938	0.1692
MBOAT1			x	41	0.0536	0.1585	0.7301
MEIS2			x	61	0.2411	0.7606	0.9361
MMP24			x	7	0.0973	0.8942	0.4405
MOBP			x	16	0.2976	0.8031	0.0657
MYT1L			x	88	0.8217	**0.0146***	0.4238
NCKAP5			x	210	0.9742	0.9654	**0.0430***
NCS1	x			20	0.9088	0.2757	0.5805
NEDD4L			x	111	0.0887	0.2316	0.6180
NOS1			x	39	0.9977	0.6423	0.3215
NRXN1			x	293	0.5215	0.5577	0.6436
NUCB1			x	5	0.5253	0.8842	0.3945
NXPH1			x	105	**0.0271***	0.5737	0.4621
PCBD1	x	x		1	0.2950	0.6027	0.2924
PPM1H			x	111	0.8022	0.4803	0.8547
PPM1L	x			38	0.3138	0.2370	0.4926
PPP1CB	x			6	0.2491	0.3061	0.4306
PPP1CC	x			4	0.8558	0.5646	0.5339
PPP1R10	x			2	0.2533	0.3078	0.5371
PPP1R11	x			1	0.6220	1	0.3181

TABLE S3.2. **GENE-BASED ASSOCIATIONS WITH VOLUME OF PUTAMEN, PALLIDUM AND CAUDATE** (CONTINUED)

ENTREZ GENE	PART OF PATHWAY			NUMBER OF SNPs	ASSOCIATION P-VALUE ($\alpha = 0.0004$)[a]		
	DOPAMINE / NOREPI-NEPHRINE	SEROTONIN	NEURITIC OUTGROWTH		PUTAMEN	PALLIDUM	CAUDATE
PPP1R12A	x			4	0.3992	0.6445	0.0681
PPP1R14B	x			1	0.1679	0.8424	0.2362
PPP1R14C	x			37	0.3007	0.3316	0.1535
PPP1R3A	x			3	0.2546	0.5501	0.2727
PPP1R7	x			7	0.9421	0.5460	0.9990
PPP2CB	x			3	0.7113	0.9669	0.8104
PPP2R1A	x			18	0.8894	0.4940	0.2981
PPP2R1B	x			2	0.3712	0.9024	0.1830
PPP2R2A	x			14	0.9721	0.4595	0.3559
PPP2R2B	x			98	0.9774	0.9531	0.2082
PPP2R2C	x			64	0.8752	0.8915	0.5457
PPP2R3A	x			12	**0.0081****	0.1717	0.5599
PPP2R3B	x			1	0.4581	0.5263	0.8316
PPP2R4	x			6	0.1163	0.0514	0.4984
PPP2R5A	x			6	0.4573	0.5243	0.3753
PPP2R5B	x			1	0.1456	0.6018	0.6820
PPP2R5C	x			18	0.6491	0.6427	0.7343
PPP2R5D	x			3	0.6599	0.5516	0.5281
PPP2R5E	x			18	0.9330	0.7733	0.8039
PRKACA	x			1	0.8175	0.1071	0.7464
PRKACB	x			17	0.1033	0.3075	0.6232
PRKAG1	x			1	0.9876	1	0.1613
PRKAG2	x			112	0.4565	0.3218	0.2608
PRKAR1A	x			4	0.3668	0.8518	0.4419
PRKAR2A	x			3	0.7470	0.6999	0.5897
PRKAR2B	x			19	0.2540	0.2458	0.5806
PTS	x	x		1	0.7574	0.9177	0.4703
QDPR	x	x		9	0.5885	0.9138	0.8738
RORA			x	304	0.2881	0.7760	0.5606
SLC18A1	x	x		18	0.4216	0.4689	0.2021
SLC18A2	x	x		19	0.5987	0.7815	0.5265
SLC18A3	x	x		1	**0.0265***	0.8077	0.8104
SLC6A2	x			32	0.7600	0.4069	0.9627
SLC6A3	x			25	0.4820	0.2624	0.7853
SLC6A4		x		10	0.5687	0.5766	0.3856
SLCO3A1			x	112	0.7373	0.2007	0.2794
SMOX	x	x		8	0.7418	0.2824	0.1562
SPOCK3			x	78	0.9644	0.7987	0.1995
SUPT3H			x	56	0.2934	0.4766	0.8849
TH	x			2	0.0970	0.1772	0.0982

TABLE S3.2. **GENE-BASED ASSOCIATIONS WITH VOLUME OF PUTAMEN, PALLIDUM AND CAUDATE** (CONTINUED)

ENTREZ GENE	PART OF PATHWAY			NUMBER OF SNPs	ASSOCIATION P-VALUE ($\alpha = 0.0004$)[a]		
	DOPAMINE / NOREPI-NEPHRINE	SEROTONIN	NEURITIC OUTGROWTH		PUTAMEN	PALLIDUM	CAUDATE
TLL2			x	40	0.4728	0.0974	0.3787
TPH1		x		4	0.7490	0.4879	0.4514
TPH2		x		28	0.4791	0.3252	0.5056
UGT1A9			x	46	0.6762	0.6650	0.7973
UNC5B			x	22	0.6130	0.6803	0.5587
ZNF423			x	98	0.0522	0.0547	0.6657

NOTE. n=344.Number of genes dopamine/norepinephrine pathway=67, number of genes serotonin pathway=28, number of genes neuritic outgrowth pathway=43. The strongest found associations are indicated: *** p-value <0.005, ** p-value between 0.005 and 0.01, * p-value between 0.01 and 0.05. [a] Corrected threshold for significance was set at p=0.0004 (0.05/126 genes).

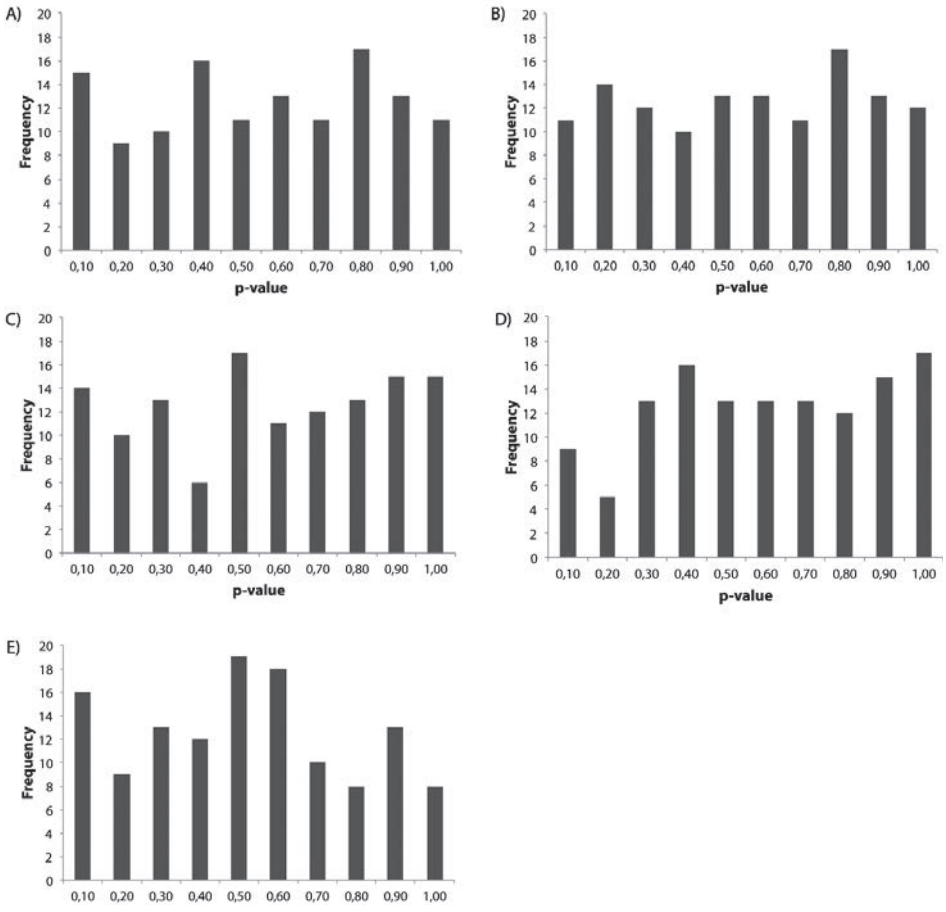

FIGURE S3.1. Frequency histograms of gene-based p-values, displaying the distribution of p-values in relation to the different (intermediate) phenotypes. A) Conners inattention score, B) Conners hyperactivity/impulsivity score, C) volume of putamen, D) volume of pallidum, E) volume of caudate.

CHAPTER

4

Gyrification differences in children and adolescents with velocardiofacial syndrome and attention-deficit/ hyperactivity disorder

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Published as Psychiatry Research Neuroimaging, 2014, 221(2), 169-171

ABSTRACT

Background

Children with Velocardiofacial Syndrome (VCFS) often present with symptoms similar to Attention-Deficit/Hyperactivity Disorder (ADHD). However, it isn't known whether similarities in underlying neurobiology exist that are related to both phenotypes. The goal of this study was to investigate patterns of gyrification between children with VCFS, ADHD, and healthy controls. We hypothesized that while VCFS and ADHD would show specific differences, notably in the parietal lobes, similar patterns of aberrant gyrification would be present in the frontal lobes.

Methods

MR images were acquired in 19 children with ADHD, 9 with VCFS, and 23 matched controls. Measures of gyrification were calculated for both hemispheres and in lobar regions.

Results

Children with VCFS showed significant decreases in gyrification compared to both children with ADHD and controls, predominantly located in the frontal, parietal, occipital lobes and the cingulate cortex. Children with ADHD showed increased gyrification in the left medial temporal lobe. There was little overlap in gyrification between the two diagnoses.

Conclusions

VCFS is associated with widespread decreases in gyrification, possibly related to a decreased brain connectivity. In ADHD we found minor differences. No evidence was found for common patterns of gyrification between VCFS and ADHD.

INTRODUCTION

Children with Velocardiofacial Syndrome (VCFS) have a considerable increased risk for developing severe psychiatric disorders during their lifetime. The first presentation of symptoms often occurs during the school age years when it is not uncommon for them to have problems with inattention, hyperactivity, and impulsivity (Shprintzen, 2000). Therefore, children with VCFS are often diagnosed with Attention-Deficit Hyperactivity Disorder (ADHD) (Antshel et al., 2007). However, while the inattention and impulsive behavior seen by some children with VCFS is characteristically similar to children with ADHD only, it's unclear if there is overlap in the neurobiology underlying this behavioral phenotype.

Magnetic resonance imaging (MRI) techniques are used to evaluate specific attributes of the underlying neurobiology of psychiatric disorders. One specific attribute that can be quantified using high-resolution structural MRI techniques is brain gyrification. Gyrification is a developmental process in which the brain undergoes changes in surface morphology to create sulcal and gyral regions (Zilles et al., 1988) and is thought to provide a measure of early neurodevelopment (Armstrong et al., 1995). The process of gyrification begins between the 10th to 15th week of gestational life. However, most sulci and gyri form during the third trimester of fetal life, during a period of rapid brain growth (Chi et al., 1977; Welker, 1990; Zilles et al., 1997).

Gyrification has played a significant role in the phylogeny of brain development, since it provides a greater surface area of the brain per unit volume and thus facilitates efficient packing of gray matter (GM) (Hilgetag and Barbas, 2006). This efficient packing of neurons likely implies a greater potential for computational abilities in the brain (Van Essen, 1997). While there is evidence from postmortem studies that quantitative measures of gyrification remain relatively constant following birth (Zilles et al., 1988; Armstrong et al., 1995), such constancy has not been seen in MRI studies of gyrification (Magnotta et al., 1999; White et al., 2010). Nevertheless, differences in gyrification suggest early aberrant patterns of neurodevelopment that may reflect underlying abnormalities in neuronal connectivity between brain regions. Aberrant connectivity could translate into less efficient neuronal processing, thus resulting in cognitive and behavioral symptoms (White and Gottesman, 2012).

There have been only a few studies that have evaluated gyrification in either VCFS or ADHD, with no studies comparing the two disorders. The studies that have evaluated gyrification in VCFS show mixed results. Studies by Schaer et al. (2006), Kunwar et al. (2012), and Srivastava et al. (2012) show a decrease in gyrification in both the frontal and parietal lobes in VCFS. However, Srivastava et al. (2012) also found that children with VCFS showed decreased gyrification in occipital and midline regions of the brain, which were not seen in the other studies. Finally, Bearden et al. (2009) found an increased surface complexity in the temporal-occipital junction in children with VCFS. Increased surface complexity would imply greater gyrification in those regions, which is contrary to the findings of decreased gyrification seen by Schaer et al. (2006), Kunwar et al. (2012) and Srivastava et al. (2012). Mixed findings in the studies could also be attributed to different methodological approaches to measure GI, as the studies used different techniques to measure GI.

Similar to VCFS, the two studies that have explored cortical folding or surface complexity in ADHD also show mixed results. Wolosin et al. (2009) studied a group of children with ADHD between 8.7 and 12.8 years of age and found a global decrease in cortical folding between children with ADHD and controls. When evaluating lobar measures and controlling for multiple testing, they found reduced folding

only in the right frontal lobe. Shaw et al. (2012) evaluated a large group of children and adolescents with ADHD using a global three-dimensional measure of gyrification and found no hemispheric differences between children with ADHD and controls.

Considering the overlap in ADHD-like symptoms in children with VCFS, coupled with the mixed results of studies of gyrification in VCFS and ADHD, the goal of this study was to explore and perform a direct comparison of gyrification between these disorders and with typically developing children as controls. Given the mixed findings of global differences in gyrification in ADHD, coupled with the findings of frontal and parietal differences in gyrification in VCFS, we hypothesized that there would be an overlap in gyrification abnormalities in the frontal lobe. However, due to pronounced difficulties with visuospatial abilities in children with VCFS (Furniss et al., 2011), we hypothesized that the parietal lobe would also show gyrification abnormalities, but only in the children with VCFS.

MATERIALS AND METHODS

Participants

This study included 19 children and adolescents with ADHD, 9 children with VCFS, and 23 age- and gender matched controls (Table 4.1). The ADHD group included 16 boys and 3 girls with a mean age of 15.4 (range 12-19), the VCFS group consisted of 5 boys and 4 girls with a mean age of 13.6 (range 10-18), and the control group included 12 boys and 11 girls with a mean age of 14.8 (range 9-19). The Hollingshead Index (1975) was used to determine Socioeconomic Status (SES) for each child in the study (Hollingshead, 1975). The ADHD and control groups represent a subgroup of subjects who participated in a larger study of the neurophysiology of attention and executive function in ADHD (Karatekin et al., 2007; Karatekin et al., 2008; Karatekin et al., 2009a, 2009b; Davenport et al., 2010; Karatekin et al., 2010a, 2010b; White et al., 2014).

ADHD participants were recruited from ADHD support groups, schools, and through advertisements in the local community. Children with VCFS were recruited from the VCFS clinic at the University of Minnesota Medical School and through advertisement in the regional parental support network. All children with VCFS had confirmed 22q11.2 deletions, using a fluorescence in situ hybridization test (FISH). The healthy volunteers were recruited through schools and advertisements in the local community.

Potential participants were excluded if they were born prematurely (< 36 weeks), had a history of significant neurological problems such as seizure disorders or a severe head injury. Potential ADHD participants were excluded if they had an IQ lower than 70 and were included only if they had, or had a history of, ADHD combined subtype. Potential controls were excluded if they had an IQ lower than 70 or evidence of academic difficulties, if they had ever taken psychoactive medications or had been diagnosed with a psychiatric disorder. Potential controls that had attention problems for which they had sought help or had a first-degree biological relative with ADHD or schizophrenia were also excluded from the study. A Kiddie-SADS (Kaufman et al., 1997) was performed on all subjects by either CK or TW to confirm the diagnosis of combined-type ADHD, determine co-occurring disorders, or to rule out Axis I diagnoses in the case of the controls.

All participants were thoroughly informed about the study procedure and provided written consent before the start of the study. In the case of minors, both parental consent and subject assent were obtained. The study was approved by the Institutional Review Board at the University of Minnesota.

Magnetic Resonance Data Acquisition

For all participants MR images were acquired using a 3Tesla Siemens MR System (Erlangen, Germany) at the Center for Magnetic Resonance Research at the University of Minnesota. The children rested comfortably in the scanner with head stabilization performed using a vacuum bag. After obtaining a localizer sequence for orientation, the high-resolution images were acquired using an MP-RAGE sequence (TR/TE = 2530/3.81, flip angle = 7, FoV 160 mm, in-plane resolution of 0.625 × 0.625 × 1.5 mm, NEX = 1) with an 8-channel head coil.

Computation of Gyrification Indices

The pre-processing of the structural imaging data was performed using the fully automated FreeSurfer software program (Massachusetts, USA, http://www.nmr.mgh.harvard.edu). Gyrification indices were computed using a novel 3D geometric approach for the automatic computation of global and regional gyrification indices (GI) from magnetic resonance images of human brains (Su et al., 2012). First, a triangular mesh of the pial surface and the hull surface of the human brain was computed. The regional surface areas for the pial and hull surfaces were calculated based on the summation of the triangular meshes within the specific region. The GI for this region of interest was calculated using the following formula:

$$\mathrm{GI}^{1}_{region} = \frac{A^{s}_{p}}{A^{s}_{h}}$$

where A^{s}_{p} is the area of any selected lobe on the pial surface and A^{s}_{h} is the corresponding area on the hull surface. We chose to use this gyrification algorithm, as it provides a regional and anatomically-based measure of GI. We predicted to find regional rather than localized differences in GI.

Statistical Analyses

All data were analyzed using SAS software version 9.2 (SAS Institute Inc., Cary, NC, USA). Demographic information was analyzed using one-way Analysis of Variance (ANOVA) for the continuous data and $\chi 2$ or Fisher's Exact Test for categorical data. Evaluation of gyrification was performed in a step-wise fashion to reduce multiple testing. Paired t-tests were used to assess whether there were global differences in GI between the right and left hemisphere. An ANCOVA was used to assess differences between global GI measures in the right and left hemisphere. If differences were found, these were followed up evaluating lobar differences. For significant findings, post-hoc testing of contrasts was performed to evaluate the ADHD group versus the controls, the VCFS group versus the controls, and the ADHD group versus the VCFS group.

A statistical threshold of $P < 0.05$ was used for interpretation and Šidák correction was applied to correct for multiple testing. Due to the high correlations between GI measures in the right and left

hemispheres (r's ranging from 0.58 to 0.94) a full Šidák correction of 12 tests would be overly strict. Thus, to account for this lack of independence, we calculated the effective number of tests (M_{eff}) that we performed, using a method described by Galwey et. al (2009), following the formula:

$$M_{eff} = \frac{\left(\sum_{i=1}^{M} \sqrt{\lambda_i}\right)^2}{\sum_{i=1}^{M} \lambda_i}$$

where M is the total number of original tests performed and λ_i are the eigenvalues of the correlation matrix i=1…M. This formula yielded an effective number of 8.56 tests. Accordingly, we adapted the Šidák correction as follows:

$$p_{min,corrected,adjusted} = 1 - (1 - p_{min})^{M_{eff}}$$

where p_{min} is the original, uncorrected, P-value and $p_{min,corrected,adjusted}$ the eventual P-value corrected for the number of effective tests performed. Uncorrected P-values are also reported. Effect sizes in the post-hoc analyses were calculated with Cohen's d (Cohen, 1988).

RESULTS

Demographics

Table 4.1 shows the demographics for the ADHD, VCFS, and control groups. There were no statistically significant differences between the groups in age, gender, ethnicity, or socioeconomic status. There was a significant difference in handedness between the groups, with the VCFS group having a significantly greater number of left-handed children (P<0.01). Estimated IQ also differed significantly between the groups, with the VCFS subjects having a significantly lower IQ (P<0.01). Data on IQ was missing in seven controls and in one VCFS subject, however we did have data on academic performance in these children. This showed that five of the controls scored superior grades (mostly A's), one scored good grades (mostly B's), and one average grades (mostly C's). The one VCFS subject scored poor grades (mostly D's).

Gyrification, Lateralization, and Sex

There was a significant difference between gyrification globally in the right versus the left hemisphere, with the right hemisphere showing a larger GI (t=-3.2, df 50, P=0.002). Within the lobar measures, the right parietal lobe (t=-8.2, df 50, P<0.0001) and cingulate cortex (t=-4.0, df 50, P=0.0002) had a greater GI, whereas the left medial temporal (t=3.3, df 50, P=0.002) and occipital (t=3.7, df 50, P=0.0006) lobes had a larger GI. There were no differences in GI between the right and left frontal lobes. Given these significant laterality differences, GI was assessed separately between the right and left lobes.

An ANCOVA of sex using age as a covariate resulted in a significant effect of sex in the right medial temporal lobe ($F_{1,48}$=5.1, P=0.03). Males had slightly larger GI in the medial temporal lobes. There was also a trend difference in GI in the left medial temporal lobe in the same direction. Due to these differences, sex was used as a covariate in the analyses.

TABLE 4.1. **DEMOGRAPHICS PARTICIPANTS**

	ADHD (n= 19)	VCFS (n= 9)	CONTROLS (n= 23)
Age (years)	15.4 (2.2)	13.6 (3.0)	14.8 (2.6)
Age range (years)	12-19	10-18	9-19
Gender			
• Female	3	4	11
• Male	16	5	12
Handedness			
• Right	17	3	18
• Left	1	**4**	1
• Ambidextrous	1	0	1
• Unknown	0	2	3
Ethnicity			
• Non-Hispanic / Non-Latino	18	8	23
• Hispanic / Latino	0	1	0
• Other	1	0	0
Parental Socioeconomic Status	52.7 (9.6)	47.7 (10.1)	50.4 (12.0)
ADHD diagnoses[a]	19	6	0
Co-occurring diagnoses (previous/current)[b]			
• Anxiety Disorders	3	2	0
• Disruptive Behavior Disorders	4	7	0
• Elimination Disorders	1	3	0
• Learning Disabilities	5	1	0
• Mood Disorders	5	2	0
• Psychosis Spectrum	0	1	0
• Substance use Disorders	5	0	0
• Other	0	1	0
Estimated IQ[c]	114.5 (13.0)	**84.6 (17.4)**	113.6 (12.6)

Values given as mean (SD) or counts. ADHD, Attention-Deficit/Hyperactivity Disorder. VCFS, Velocardiofacial Syndrome. [a] Primary diagnosis in ADHD group, co-occurring diagnosis in VCFS group. [b] Numbers reflect counts of co-occurring diagnoses and thus do not add up to group totals since children can have no or multiple co-occurring diagnoses. [c] IQ was estimated from the Vocabulary and Block Design subtests from the Wechsler Intelligence Scale, 3rd ed. (WISC-III; Wechsler, 1991), the WISC-IV (Wechsler, 2003), or the Wechsler Adult Intelligence Scale, 3rd ed. (Wechsler, 1997). Data on IQ was missing in 7 controls and 1 VCFS subject. Significant results highlighted in **bold**.

Gyrification Index in VCFS, ADHD, and Controls

Table 4.2 shows the results of the 3 (diagnosis) by 2 (sex) ANCOVA analyses of gyrification with age as a covariate. The ANCOVA showed significant differences in both the left ($F_{2,46}$=7.2, P=0.002) and right ($F_{2,46}$=5.0, P=0.01) global measures of gyrification. Next, we explored the right and left lobar measures separately. A 3 (diagnosis) by 2 (sex) ANCOVA with age as a covariate showed group differences in the left frontal ($F_{2,46}$=9.0, P=0.0005), left medial temporal ($F_{2,46}$=3.3, P=0.04), and the left occipital lobes ($F_{2,46}$=9.4, P=0.0004). The left frontal and occipital lobes survived correction for multiple comparisons. In the right hemisphere, a 3 (diagnosis) by 2 (sex) ANCOVA with age as a covariate showed group differ-

ences in the right frontal ($F_{2,46}$=5.3, P=0.008), right parietal ($F_{2,46}$=5.9, P=0.005), right occipital ($F_{2,46}$=3.2, P=0.05), and the right cingulate ($F_{2,46}$=6.0, P=0.005). The right parietal lobe and the cingulate cortex survived correction for multiple testing.

TABLE 4.2. **ANCOVA'S GYRIFICATION INDEX**

ROI	ADHD[a]	VCFS[a]	CONTROLS[a]	F	P	P_{CORR}[b]
GLOBAL MEASURES						
Left	2.66 (2.61-2.70)	2.54 (2.48-2.60)	2.66 (2.62-2.69)	7.15	**0.002**	
Right	2.68 (2.63-2.72)	2.57 (2.51-2.62)	2.66 (2.62-2.69)	5.05	**0.01**	
LOBAR MEASURES						
Left Frontal	2.48 (2.43-2.53)	2.34 (2.28-2.40)	2.49 (2.45-2.52)	9.04	**0.0005**	**0.004**
Right Frontal	2.50 (2.45-2.56)	2.37 (2.29-2.44)	2.48 (2.44-2.53)	5.32	**0.008**	0.07
Left Parietal	3.12 (3.06-3.19)	2.99 (2.90-3.08)	3.10 (3.04-3.15)	3.11	0.05	0.38
Right Parietal	3.26 (3.20-3.33)	3.08 (3.00-3.16)	3.20 (3.15-3.25)	5.90	**0.005**	**0.04**
Left Temporal	2.65 (2.58-2.73)	2.62 (2.53-2.72)	2.69 (2.63-2.75)	0.83	0.44	0.99
Right Temporal	2.67 (2.60-2.74)	2.67 (2.58-2.76)	2.68 (2.62-2.73)	0.01	0.99	1.00
Left Medial Temporal	2.16 (2.08-2.23)	2.03 (1.94-2.13)	2.05 (1.99-2.11)	3.33	**0.04**	0.32
Right Medial Temporal	1.99 (1.90-2.08)	2.00 (1.88-2.12)	1.98 (1.90-2.05)	0.06	0.94	1.00
Left Occipital	2.53 (2.48-2.59)	2.36 (2.29-2.43)	2.53 (2.48-2.57)	9.38	**0.0004**	**0.003**
Right Occipital	2.49 (2.43-2.55)	2.36 (2.28-2.44)	2.43 (2.38-2.48)	3.22	**0.05**	0.35
Left Cingulate	1.90 (1.85-1.96)	1.77 (1.68-1.86)	1.90 (1.85-1.96)	3.16	0.05	0.37
Right Cingulate	1.96 (1.87-2.05)	1.78 (1.66-1.90)	2.02 (1.95-2.09)	6.03	**0.005**	**0.04**

[a] Adjusted (estimated marginal) means and 95% confidence intervals are shown. [b] Šidák corrected P-values for the effective number of tests. ADHD, Attention-Deficit/Hyperactivity Disorder. VCFS, Velocardiofacial Syndrome. Significant results highlighted in **bold**.

Gyrification Index ADHD Versus Controls

Table 4.3 shows the results of the post-hoc ANCOVAs to assess differences between the ADHD and the control group. A 2 (diagnosis) by 2 (sex) ANCOVA of GI with age as a covariate only showed an effect of diagnosis in the left medial temporal lobe ($F_{1,38}$=7.2, P=0.01, d=0.88). In this lobe, the children with ADHD showed a greater GI compared to the controls. For the other lobes, GI was similar in ADHD and controls. Since boys were overrepresented in the ADHD group compared to the controls and since gender may have an effect on the results, we also performed the analyses in boys only. This yielded similar results. The only difference was an additional significant finding in the right occipital lobe, with ADHD showing a higher GI. In these gender-stratified analyses the p-value changed from 0.26 (in the original analyses) to 0.04. Since the ADHD literature shows age-related differences, we also evaluated whether there was an age-by-diagnosis interaction. We found an age-by-diagnosis interaction in the left cingulate cortex ($F_{1,37}$=10.4, P=0.003) with the younger ADHD children showing a nominally smaller left cingulate GI compared to controls, which disappears in the older ADHD children. The right cingulate cortex showed similar findings, at a trend level ($F_{1,37}$=3.8, P=0.06).

Gyrification Index VCFS versus Controls

Table 4.3 shows the results of the post-hoc ANCOVAs to assess the differences between VCFS and the control group. A 2 (diagnosis) by 2 (sex) ANCOVA analysis of GI with age as a covariate showed significant lower GI in VCFS children in both the left ($F_{1,28}$=12.4, P=0.002, d=1.12) and right ($F_{1,28}$=6.9, P=0.01, d=0.84) global measures of gyrification. On a lobar level we found lower GI in VCFS children in the left frontal ($F_{1,28}$=15.0, P=0.0006, d=1.22), left parietal ($F_{1,28}$=4.7, P=0.04, d=0.66), left occipital ($F_{1,28}$=16.1, P=0.0004, d=1.62), and the left cingulate cortex ($F_{1,28}$=11.7, P=0.002, d=1.33). In the right hemisphere, ANCOVAs showed that children with VCFS had lower GI in the right frontal ($F_{1,28}$=6.2, P=0.02, d=0.77), right parietal ($F_{1,28}$=6.0, P=0.02, d=0.77), and the right cingulate cortex ($F_{1,28}$=10.8, P=0.003, d=1.26).
We additionally found an age-by-diagnosis interaction in the left cingulate cortex ($F_{1,27}$=9.0, P=0.006), which was similar to the interaction in this lobe between ADHD and controls. The younger VCFS children showed a smaller left cingulate GI compared to controls, which disappears in the older VCFS children. We also found an age-by-diagnosis interaction in the right medial temporal lobe ($F_{1,27}$=5.2, P=0.03), with the older VCFS children showing a higher GI than the young VCFS children, while the controls showed a decrease in GI with age.

Gyrification Index ADHD versus VCFS

Table 4.3 shows the results of the post-hoc ANCOVAs to assess the differences between VCFS and the ADHD group. A 2 (diagnosis) by 2 (sex) ANCOVA analysis of GI with age as a covariate showed significant lower GI in VCFS children compared to ADHD children in both the left ($F_{1,24}$=9.3, P=0.006, d=1.24) and right ($F_{1,24}$=10.6, P=0.003, d=1.29) global measures of gyrification. On a lobar level we found lower GI in VCFS children compared to ADHD children in the left frontal ($F_{1,24}$=15.5, P=0.0006, d=1.54), left parietal ($F_{1,24}$=4.8, P=0.04, d=0.71), and the left occipital ($F_{1,24}$=11.2, P=0.003, d=1.63) lobes. In the right hemisphere, the ANCOVAs showed that children with VCFS had lower GI in the right frontal ($F_{1,24}$=18.8, P=0.0002, d=1.46), right parietal ($F_{1,24}$=13.2, P=0.001, d=1.08), right occipital ($F_{1,24}$=6.3, P=0.02, d=1.11) and the right cingulate cortex ($F_{1,24}$=5.0, P=0.03, d=1.30).

We found no age-by-diagnosis interactions between ADHD and VCFS.

TABLE 4.3. **POST-HOC ANALYSES ANCOVA'S GYRIFICATION INDEX**

	ADHD VS CONTROLS			VCFS VS CONTROLS			ADHD VS VCFS		
ROI	F	P	EFFECT SIZE[a]	F	P	EFFECT SIZE[a]	F	P	EFFECT SIZE[a]
GLOBAL MEASURES									
Left	0.00	0.97	0.08	12.41	**0.002**	1.12	9.27	**0.006**	1.24
Right	0.34	0.57	0.19	6.87	**0.01**	0.84	10.56	**0.003**	1.29
LOBAR MEASURES									
Left Frontal	0.05	0.83	0.12	15.03	**0.0006**	1.22	15.52	**0.0006**	1.54
Right Frontal	0.27	0.61	0.06	6.17	**0.02**	0.77	18.84	**0.0002**	1.46
Left Parietal	0.44	0.51	0.15	4.68	**0.04**	0.66	4.84	**0.04**	0.71
Right Parietal	1.49	0.23	0.36	6.04	**0.02**	0.77	13.20	**0.001**	1.08
Left Temporal	0.14	0.71	0.33	1.18	0.29	0.31	0.11	0.74	0.00
Right Temporal	0.03	0.86	0.07	0.01	0.93	0.00	0.02	0.88	0.07
Left Medial Temporal	7.20	**0.01**	0.88	0.10	0.76	0.04	2.14	0.16	0.67
Right Medial Temporal	0.22	0.64	0.26	0.04	0.85	0.17	0.31	0.58	0.09
Left Occipital	0.22	0.64	0.03	16.11	**0.0004**	1.62	11.25	**0.003**	1.63
Right Occipital	1.31	0.26	0.47	2.05	0.16	0.52	6.34	**0.02**	1.11
Left Cingulate	0.00	0.96	0.02	11.72	**0.002**	1.33	1.88	0.18	0.92
Right Cingulate	0.48	0.49	0.26	10.81	**0.003**	1.26	5.01	**0.03**	1.30

ADHD, Attention-Deficit/Hyperactivity Disorder. VCFS, Velocardiofacial Syndrome. [a] Effect sizes presented as Cohen's *d*. Significant results highlighted in **bold**.

DISCUSSION

Our data showed that children with VCFS had significantly less gyrification in a number of brain regions compared to both children with ADHD and typically developing children. While the decrease in GI appears to be global in the VCFS children, certain brain regions appear to show greater differences than others (Figure 4.1). Children with VCFS demonstrated decreased GI in the left and right frontal and parietal lobes, left and right cingulate cortex, and the left occipital lobe compared to typically developing children. Compared to children with ADHD, children with VCFS showed bilateral decreases in the frontal, parietal, and occipital lobes, as well as the right cingulate cortex. No differences in GI in the temporal lobes were seen between VCFS children, controls, or children with ADHD. We found little evidence for an overlap between GI measures in the ADHD and VCFS children, with the VCFS children showing characteristically greater differences compared to controls and children with ADHD.

Gyrification provides a unique measure of neurodevelopment. The vast majority of gyrification occurs during the third trimester of fetal life. Furthermore, there is evidence from postmortem studies that GI remains relatively stable following birth (Armstrong et al., 1995), although MRI measures have not shown such stability (White et al., 2010; Srivastava et al., 2012). A theory underlying the formation of gyrification links this neurodevelopmental process to underlying brain connectivity, and thus overall efficiency of brain function (Van Essen, 1997; White and Gottesman, 2012). Thus, aberrant gyrification could be considered as a decrease in the overall efficiency of brain function. A decrease in efficiency of brain function

could translate into difficulties with attention and impulsive behavior. Indeed, gyrification abnormalities have been reported separately in both VCFS (Schaer et al., 2006; Kunwar et al., 2012; Srivastava et al., 2012) and ADHD (Wolosin et al., 2009). Since VCFS has been associated with a decrease in GM volume and cortical thickness (Bearden et al., 2007; Tan et al., 2009), differences in gyrification could be driven by morphological alterations secondary to synaptic pruning. This is certainly possible and is an interesting hypothesis to test since the tension based hypothesis of cortical gyrification posits that processes which decrease the tension between brain regions (i.e., pruning), would subsequently result in alterations of gyrification (Van Essen, 1997). Alternatively, decreased GM does not necessarily imply a decrease in GI, as GI is a unit-less measure. Thus, a smaller brain may have less GM yet more fissures and folds, and thus a greater GI.

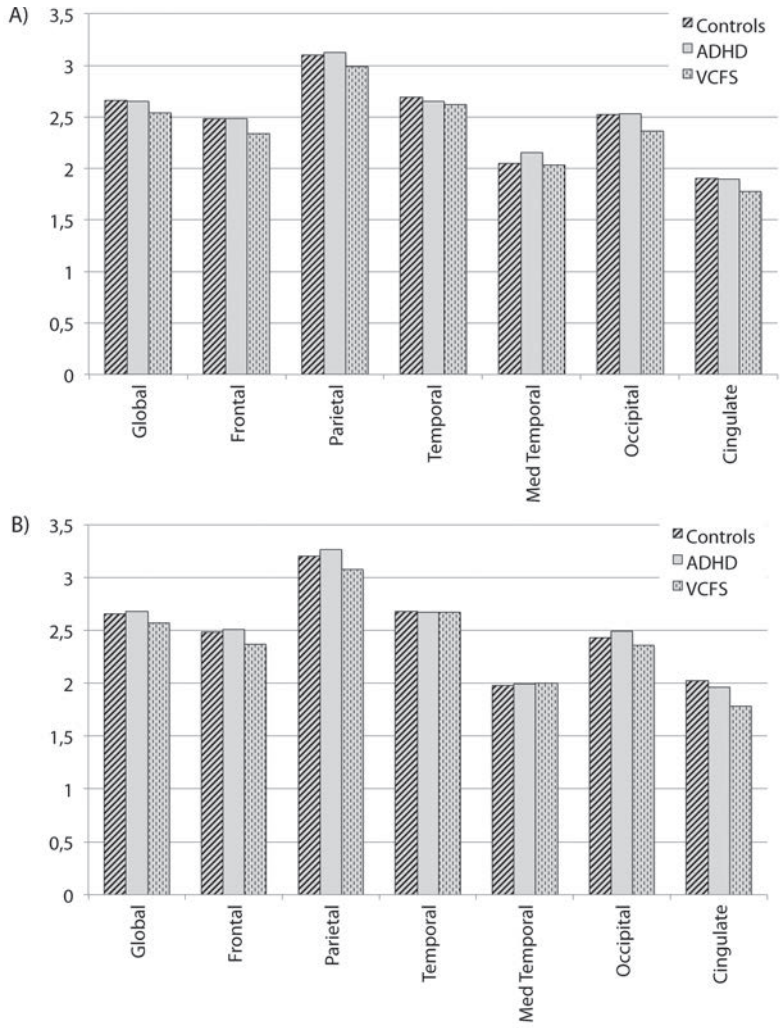

FIGURE 4.1. Estimated Marginal Means of the GI of the Left (A) and Right (B) Hemispheres in the Control, ADHD and VCFS Groups.

Patients with VCFS have characteristic symptoms that are also associated with ADHD, namely difficulties with attention, hyperactivity, and impulsivity (Shprintzen, 2000). Thus, we expected greater overlap in the neurobiological abnormalities between these two disorders. However, our findings did not confirm our expectations.

Similar to our findings, Schaer et al. (2006) found decreased GI in VCFS in the frontal and parietal lobes, bilaterally. Unlike our study, they did not find differences in the occipital lobe. However, reports of differences in surface morphology in the occipital lobe in VCFS have been described before (Bearden et al., 2009). We also showed significant decreases in the GI in the right and left cingulate cortex in children and adolescents with VCFS, which has not been reported previously.

In the children and adolescents with ADHD, we found an increased GI in the left medial temporal lobe. Interestingly, a delay in maturation of the cortex has been described in ADHD (Shaw et al., 2007). GI measures have been shown to decrease with development (White et al., 2010), thus one explanation could be that there is a delayed maturation in the left medial temporal lobe, which results in a relative increase in GI. However, to actually study a delay in maturation in ADHD, longitudinal data would be needed.

We also found an age-by-diagnosis interaction in the cingulate cortices in the ADHD group, with younger children showing a smaller cingulate GI compared to controls, which disappears in the older ADHD children. However, these cross-sectional findings should be replicated with a longitudinal design to more accurately delineate age-related effects.

A major limitation of the present study is the small sample of children with VCFS. However, in spite of the small sample size, statistically significant differences were seen in GI between the VCFS children compared to both typically developing controls and children with ADHD. Also, the effect sizes of the differences were large. Furthermore, we utilized a three-dimensional gyrification measure that is anatomically based, which may provide greater power to detect differences. Finally, there is overlap with our findings and the findings of other studies of GI with VCFS in the literature. Another limitation is the fact that all children were only scanned at one time point, thus we do not have longitudinal data. It would be interesting to determine developmental trajectories of gyrification to be able to assess when during the course of development, the differences emerged. Finally, although the age-range for recruitment was the same for each of the groups, the age range of participants in the ADHD group was smaller (12-19) compared to the VCFS (10-18) and control (9-19) groups. This might reflect a recruitment bias.

In summary, we used a novel three-dimensional geometric approach for the automatic computation of global and regional gyrification indices (Su et al., 2012) and found a global decrease in gyrification in both hemispheres in a small sample of children with VCFS. Evaluating regional measures, the decrease in GI was predominantly located in the frontal, parietal, and occipital lobes and the cingulate cortex. In children with ADHD we found a region of increased gyrification in the left medial temporal lobe. We found no evidence for a common pattern of brain gyrification between ADHD and VCFS. This suggests that there is no clear relationship between gyrification and attentional deficits that are present in both disorders. This finding could indicate that the shared symptoms between the two disorders are caused by a different underlying neurobiology, however it remains possible that there are other shared neurobiological causes, not measured with GI indices, that underly the two disorders. This knowledge might be valuable for targeting therapeutic interventions on attention problems in ADHD and VCFS.

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CHAPTER

5

The association of gender, age and intelligence with neuropsychological functioning in young typically developing children

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Applied Neuropsychology: Child, accepted for publication

ABSTRACT

Background

Although early childhood is a period of rapid neurocognitive development, few studies have assessed neuropsychological functioning in young typically developing children. Also, results regarding the association with gender and intelligence are mixed.

Methods

In 853 typically developing children, the association of gender, age and intelligence with neuropsychological functioning on the domains attention, executive functioning, language, memory, sensorimotor functioning and visuospatial processing was explored.

Results

Strong positive associations with age were observed. In addition, clear gender differences were found, showing that girls generally outperformed boys, with the exception of visuospatial tasks. Furthermore, IQ was positively associated with neuropsychological functioning, which was strongest in visuospatial tasks.

Conclusions

Performance in different neuropsychological domains is associated with age, gender and intelligence in young typically developing children.

INTRODUCTION

Although early childhood is a period of major neurocognitive development (Casey, Tottenham, Liston, & Durston, 2005; Giedd et al., 1999; Gogtay et al., 2004), relatively few studies have focused on neuropsychological functioning in young typically developing children. However, examining children's cognitive abilities during a young age is of great importance, since understanding typical development will also help us to better understand aberrant (cognitive) development in young children. In addition, previous studies have shown mixed results regarding the association of gender and intelligence with neuropsychological functioning. Therefore, the purpose of this study is to evaluate neuropsychological functioning (and specifically age-, gender- and intelligence related differences) in a large sample of typically developing children. By focusing on a narrow age range of 6 to 10 years, we present an overview of neuropsychological functioning during this important period of cognitive development.

Neuropsychological functioning is a broad concept that comprises different cognitive functions, including language, memory, executive functioning, visuospatial processing and sensory and motor functions, which are essential in daily life. These neuropsychological functions have been shown to develop at different ages and to follow different developmental trajectories. For example, simple language functions have been shown to be established at a young age, even before school-age, while more complex language functions continue to develop throughout adolescence (Korkman, Barron-Linnankoski, & Lahti-Nuuttila, 1999; Korkman, Kemp, & Kirk, 2001; Rosselli, Ardila, Navarrete, & Matute, 2010). Primary motor functions also mature early in development (before the age of 9) (Del Giudice et al., 2000; Korkman et al., 2001), whereas more complex visuospatial abilities appear to reach mastery at a later age, around the beginning of adolescence (Del Giudice et al., 2000; Korkman, Lahti-Nuuttila, Laasonen, Kemp, & Holdnack, 2013; Rosselli et al., 2010). The finding that simple motor functions develop relatively early in life is in line with findings of brain imaging studies showing that the primary sensorimotor areas (pre- and postcentral gyrus) are among the first to mature (Casey et al., 2005; Gogtay et al., 2004; Shaw et al., 2008). The prefrontal cortex, on the other hand, matures at a later age and even continues to develop well into adolescence and early adulthood (Giedd et al., 1999). Numerous studies have focused on the development of the executive functions that are mediated by the frontal regions of the brain, such as inhibition, planning, shifting and working memory. These studies reported mixed results with respect to the age at which peak performance is reached, dependent on the kind of executive function studied. However, overall it seems that most complex executive functions continue to develop throughout childhood and into young adulthood (Anderson, Anderson, Northam, Jacobs, & Catroppa, 2001; Huizinga, Dolan, & van der Molen, 2006; Korkman et al., 2013; Rosselli et al., 2010). Finally, it has been shown that most memory functions are still developing into adolescence, although the exact age of mastery varies depending on the type of memory task used and the cognitive load (Huizinga et al., 2006; Korkman et al., 2001; Korkman et al., 2013; White, Schmidt, & Karatekin, 2010).

Many studies have shown gender differences in the performance of specific neuropsychological tasks. Generally, girls outperform boys on language and other verbal tasks, while boys tend to perform better on tasks that require spatial abilities (Levine, Huttenlocher, Taylor, & Langrock, 1999; Linn & Petersen, 1985; Mann, Sasanuma, Sakuma, & Masaki, 1990; Strand, Deary, & Smith, 2006; Voyer, 2011). However, contrasting results have been reported as well (Ardila, Rosselli, Matute, & Inozemtseva, 2011; Strand et al., 2006). Surprisingly, previous publications of the authors of the NEPSY(-II), the instrument

used in our study, have not reported on the relationship between gender and neuropsychological functioning on the NEPSY-II (Korkman et al., 2001; Korkman et al., 2013).

Previous studies (mainly in adults or adolescents) have shown various neuropsychological functions to be substantially related to general intelligence (Diaz-Asper, Schretlen, & Pearlson, 2004; Jung, Yeo, Chiulli, Sibbitt, & Brooks, 2000; Seidenberg, Giordani, Berent, & Boll, 1983). However, results are mixed with regard to the strength of the association for different neuropsychological domains, and multiple studies pointed out that not all different neuropsychological functions can be explained equally well by intelligence. Some studies have shown that measures requiring problem-solving abilities and language skills (Seidenberg et al., 1983) or verbal fluency (Ardila, Pineda, & Rosselli, 2000) are more strongly related to general intelligence than simple perceptual and motor functions (Seidenberg et al., 1983) and executive functions such as response inhibition (Ardila et al., 2000). A study by Friedman et al. (2006) for example showed intelligence to be strongly related to updating working memory, but not to response inhibition and shifting (Friedman et al., 2006). However, contrasting results have been published as well (Arffa, 2007). Multiple studies have stated that intelligence tests do not measure all different cognitive functions equally well, and that neuropsychological instruments sensitive to more specific cognitive (mainly executive) functions are therefore of great importance (Ardila, 1999; Ardila et al., 2000; Friedman et al., 2006).

Since previous studies have shown mixed results regarding the role of gender and intelligence in neuropsychological functioning and since not many studies have been done in young, typically developing children, the goal of this study was to assess the association of age, gender and intelligence with neuropsychological functioning on the NEPSY-II-NL in a large group (n=853) of typically developing children between 6 and 10 years of age. In order to better understand aberrant (cognitive) development, it is of great importance to gain insight in normal development. With respect to age differences we hypothesize that, while most cognitive domains will support ongoing development, simple (visuo) motor functions will be mastered within the age range of our sample. With respect to gender differences we expect to find that girls outperform boys on language tasks, while boys will perform better on tasks requiring visuospatial abilities. With respect to the association between neuropsychological functioning and intelligence we hypothesize that, while performance on most neuropsychological tasks will show a strong association with intelligence, performance in measures of executive functioning (and in particular response inhibition) will show the weakest association with intelligence in this age group.

MATERIALS AND METHODS

Participants

This study is embedded within the Generation R study, a multi-ethnic population-based cohort, investigating children's health, growth, and development from fetal life onwards in Rotterdam, the Netherlands. An overview of the Generation R study design and population has been previously described (Jaddoe et al., 2012; Tiemeier et al., 2012).

When the children were between 6 and 10 years of age, a detailed neuropsychological assessment was performed in a subgroup of the entire Generation R population, as part of a pilot brain Magnetic

Resonance Imaging (MRI) study (White et al., 2013). Between September 2009 and July 2013, a total of 1,325 children were recruited of which 1,307 children completed the neuropsychological assessment. The neuropsychological assessment was added to the existing research protocol in March 2010 and in addition, some participants arrived late at the research center, resulting in missing neuropsychological data in 18 children. In order to focus on neuropsychological functioning in children without behavioral problems, we excluded boys and girls with a Child Behavior Checklist (CBCL 1,5-5) (Achenbach & Rescorla, 2000) score above the clinical range (syndrome and DSM-oriented scale scores > 98th percentile and broadband scale scores > 91st percentile). This resulted in a final study population of 853 children (Figure 5.1).

Demographic information such as date of birth, gender and birth weight was obtained from midwives and hospital registries. Child ethnicity was defined according to the ethnicity categorization of Statistics Netherlands (Statistics Netherlands, 2004a). Children with both parents born in the Netherlands were considered Dutch and children were classified as non-Dutch (further categorized as 'other Western' and 'other non-Western') if one parent was born outside the Netherlands. Child Behavior Checklist (CBCL) scores were obtained using a questionnaire, filled out by the primary caregiver during the assessment wave at 6 years of age. Maternal educational level was defined as highest education completed, according to the definition of Statistics Netherlands (Statistics Netherlands, 2004b) and household income was defined by the total net monthly income of the household. Information on maternal smoking and alcohol use during pregnancy was obtained using questionnaires in each trimester of pregnancy. Child and maternal characteristics are shown in Table 5.1.

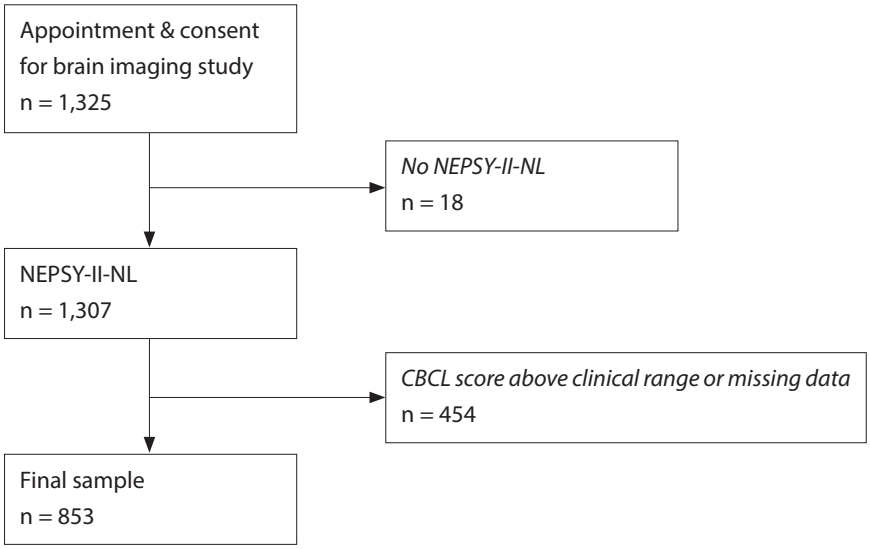

FIGURE 5.1. Flowchart participant selection

TABLE 5.1. **CHILD AND MATERNAL CHARACTERISTICS (n=853)**

	MEAN (SD)
CHILD CHARACTERISTICS	
Gender, % boys	51.2
Mean age during NEPSY-II-NL assessment (yr.;mo.)	7;10
SON-R nonverbal IQ (score)[a]	104.0 (13.5)
• Below average IQ (<90), %	13.7
• Average IQ (90-110), %	55.2
• Above average IQ (>110), %	31.1
Mean age during SON-R IQ assessment (yr.;mo.)[a]	6;1
Ethnicity, %	
• Dutch	74.4
• Other Western	8.1
• Non Western	17.5
Child Behavior Checklist (score)	
• Total score	17.5 (11.9)
• Internalizing problems	5.0 (3.9)
• Externalizing problems	7.0 (5.6)
Birth weight (grams)	3442.3 (571.6)
MATERNAL CHARACTERISTICS	
Educational level, %	
• High	57.9
• Medium	30.7
• Low	10.1
Monthly household income, %	
• > €2000	75.0
• €1200 - €2000	14.3
• < €1200	4.8
Smoking (any), %	
• Never in pregnancy	76.1
• Until pregnancy was known	7.0
• Continued in pregnancy	14.8
Alcohol use (any), %	
• Never in pregnancy	32.2
• Until pregnancy was known	13.4
• Continued in pregnancy	48.8

NOTE. Values represent mean (SD), unless otherwise indicated. [a] n with IQ data =679.

Neuropsychological functioning

The neuropsychological assessment was performed using the NEPSY-II-NL. The NEPSY-II-NL is a Dutch translation and adaptation of the North American NEPSY-II (Brooks, Sherman, & Strauss, 2010). The NEPSY-II-NL can be used to assess neuropsychological functioning in 5-to-12 year-old children. To our

knowledge this is the first study using the Dutch translation/adaptation of the original NEPSY-II. The full NEPSY-II-NL battery consists of 34 tasks (Korkman et al., 2010b). Due to time constraints, we selected a battery of ten tasks from the NEPSY-II-NL (White et al., 2013). Each of these ten task falls into five specific NEPSY-II-NL neuropsychological domains: Attention and Executive Functioning, Language, Memory and Learning, Sensorimotor Functioning, and Visuospatial Processing. The battery took approximately 55 minutes to administer and the children were randomly assigned to receive one of four selected orders of task administration. A smaller battery of 6 NEPSY-II-NL tasks was assessed in a subgroup of our study population, resulting in a high number of missings for 4 tasks (Statue, Narrative Memory, Geometric Puzzles and Route Finding).

Rules from the manual of the NEPSY-II-NL were closely followed (Korkman, Kirk, & Kemp, 2010a). These rules described start procedures (e.g. older children may start with different items than younger children) and stop procedures (e.g. after 5 subsequent scores of 0 a certain task may be stopped). However, in order to fully explore age effects, we did not follow age-related stop procedures (sometimes younger children were allowed to stop a task earlier than older children). Finally, children with incomplete or unreliable (as observed by the test assistant) data due to lack of cooperation for any individual NEPSY-II-NL task were excluded from the analyses.

Attention and Executive Functioning

Multiple interrelated processes define the neuropsychological constructs attention and executive functions. We used two different tasks of the attention and executive functioning domain of the NEPSY-II-NL. The first task was the Auditory Attention and Response Set task, which consists of two parts. The Auditory Attention component was administered first and measures selective and sustained attention. Selective attention refers to the ability to focus on a specific task while suppressing irrelevant stimuli. Sustained attention refers to the ability to attend to a task for a long(er) period of time. In the Auditory Attention task the children were presented recordings of a long list of color- and other words and asked to only respond to the word 'Red' by touching the red circle on the sheet in front of them. The sheet also contained a blue, black, and yellow circle, but these had to be ignored. Touching the red circle within 2 seconds indicates a correct response.

Following the Auditory Attention component, Response Set was performed. This task taps into response inhibition and working memory. Inhibition is the ability to suppress (automatic) behavior. Working memory is required to keep information actively in mind for as long as needed to complete a task. In this task, children must respond to the word 'Red' by touching the yellow circle, respond to 'Yellow' by touching the red circle and lastly, respond to the word 'Blue' by touching the blue circle. All the other colors or words should be ignored. Touching the correct circle within 2 seconds indicates a correct response. Touching another color is incorrect, as well as having a delayed response (not within 2-second interval). Even though children younger than 7 years of age should stop after the first task (i.e. Auditory attention) according to the NEPSY-II-NL manual (Korkman et al., 2010a), Response Set was assessed in all participants, including the 6-year-old children. From the Auditory Attention and Response Set task various summary scores were calculated. These included the total correct responses and the total number of commission, omission, and inhibition errors.

The second task in the domain Attention and Executive Functioning is the Statue task. This task requires a child to maintain a 'statue-like' body position for a period of 75 seconds, while at the same

time ignoring environmental distractors. This task measures motor persistence and response inhibition during 15 intervals of 5 seconds each. Summary measures from the Statue task include the total number of body movements, eye openings, sound productions, and a total score. According to the NEPSY-II-NL manual, this task is only suitable for children up to and including 6 years of age (Korkman et al., 2010b). Therefore, we performed the analyses only in children of 6 years of age, in order to prevent a ceiling effect.

Language

The language skills domain involved a test of verbal fluency, the Word Generation task. This task measures how many words a child can generate within 60 seconds in two semantic categories. In the first category children have to name as many animals as possible and in the second category food and drinks. The total semantic score is the sum of the total number of correctly generated words for both categories together. Correct words include existing words, are not proper nouns, and have not been mentioned before by the child (no repetitions).

Memory and Learning

The memory and learning domain included an immediate and delayed memory for faces task and a verbal memory task. During the Memory for Faces task the child was first presented with multiple series of three faces and asked to look closely at each face (for 5 seconds). The child was then provided with another set of three faces and was asked which face he or she had seen before. Immediate recall is the skill to retrieve information from memory immediately after learning. The delayed recall version of this task was assessed after a delay period of 15 to 25 minutes and measured the ability to retrieve information after a longer period of time. All presented faces showed a neutral expression. A total correct score was calculated for both the immediate and delayed recall.

We used the Narrative Memory task to assess verbal memory, specifically immediate free recall, cued recall, and (passive) recognition of verbal information. In this task, children listened to a short story after which the child was asked to provide as many details about the story as he or she could remember. This free recall component of the task measures the child's ability to remember and actively recall the story. Subsequently, children were asked specific questions about the story (cued recall), and finally questions that only required yes and no answers and/or multiple-choice questions (recognition). The Narrative Memory task provides a total correct score for the free and cued recall combined, the free recall only, and for recognition.

Sensorimotor Functioning

To gain motor control, one has to be able to combine motor activity and sensory feedback. For example, visuomotor accuracy requires visual input and motor output. During the paper-and-pencil task Visuomotor Precision, the child draws a line with the dominant hand as quickly and as accurately as possible along a paper path. The paper path consists of a set of parallel curved lines and the child was asked to draw a line, as quickly and with as few errors as possible, in-between the two lines. Summary scores for the Visuomotor Precision task include the total completion time, total number of errors (i.e. drawing outside the lines of the path), and the total number of times that the child lifted the pencil. These summary scores tap into both the speed and accuracy of visuomotor performance.

Visuospatial Processing

Visuospatial processing refers to the neuropsychological constructs of visual perception and spatial processing. Matching visual patterns and identifying figures within a picture are examples of visual perception skills, whereas mental rotation and judging orientation and direction are examples of spatial processing skills. The visuospatial processing domain consisted of three different tasks.

The Arrows task measured the child's ability to judge the direction of an arrow by asking the child to select, out of multiple arrows, the correct arrow(s) that point(s) to center of a target. The summary score for the Arrows task is the total number of correct responses.

The Geometric Puzzles task measured mental rotation, visuospatial working memory, and attention to detail. This task requires a child to discriminate which abstract figures in a set match those within a grid containing multiple abstract figures. Figures in the grid can be rotated and thus be not exactly the same as the example figure. Even though the NEPSY-II-NL manual states that children of 6 years or younger should stop after completion of 12 items (Korkman et al., 2010a), the whole task (of 20 items) was assessed in all participants, regardless of age.

Finally, we administered the Route Finding task, which measures visuospatial relations, orientation, and direction. The child used a skeleton map showing a specific route to a house and needs to translate this route onto a map containing houses and side streets. The maps progress from simple to complex. The summary score obtained from this task is the total correct score from a series of 10 maps.

Intelligence

IQ of the child was assessed during the assessment wave at 6 years of age, using a shortened version of the Snijders-Oomen Niet-verbale intelligentie Test – Revisie (SON-R 2.5–7). The SON-R 2.5-7 is a non-verbal intelligence test suited for children of 2.5–7 years of age (Tellegen, Winkel, Wijnberg-Williams, & Laros, 2005). Data on intelligence was available in 679 of the 853 children in total.

Statistical analyses

To analyze the association of gender with the NEPSY-II-NL scores, we performed a two-way analysis of variance (ANOVA). To assess the association of age and intelligence with neuropsychological functioning, we performed linear regression analyses. All analyses were adjusted for child ethnicity. Analyses of age differences were additionally adjusted for gender and vice versa. Analyses on intelligence were additionally adjusted for both age and gender.

In the analyses of age differences, we also tested a model with a quadratic age-term (age in years squared), in order to explore potential non-linear age associations and to assess potential plateau-effects in performance, which could represent the age of mastery of a certain neuropsychological function. If a non-linear age association was found, effect estimates (both the linear and quadratic) of the quadratic model (that included the squared term) were provided in the text and Table 5.2. If there was no non-linear effect, the effect estimate of the linear model was provided. For ease of interpretation, visualization and to examine whether and in which age range mastery took place, we additionally examined age in seven age groups in relation to neuropsychological performance. We used the oldest age group as a reference category in these analyses (Figure 5.3).

For summary scores that were not normally distributed we applied either square root or log transformations were applied to approach a normal distribution. All analyses were performed using SPSS Statistics version 21.

RESULTS

For all neuropsychological tasks, the results of the analyses of age associations are shown in Table 5.2. Table 5.3 provides the results of the analyses regarding gender differences. In addition, Figure 5.2 provides a visual representation of the effect of gender and age on neuropsychological functioning. In Figure 5.3 non-linear age associations are depicted.

For the association between intelligence and neuropsychological functioning, the results of the regression analyses are summarized in Table 5.4. Since some scores were mathematically transformed in the regression analyses, in Table 5.5 we also provided partial correlations using the original, untransformed variables (and adjusted for child age, gender and ethnicity) in order to be able to assess the strength of the association.

Attention and Executive Functioning

A total of 834 children completed the Auditory Attention task. The analyses show that older children had a higher total score than younger children ($\beta = 0.18$, $p < 0.0001$). For the amount of commission errors, we found a non-linear association, potentially indicating a plateau-effect of performance with age ($\beta = 0.08$, $p = 0.026$). Figure 5.3 shows this non-linear relationship, indicating a reduction of commission errors that remained relatively stable from the age range 7-5-8 years onwards. Older children also made fewer omission ($\beta = -0.34$, $p < 0.0001$) and inhibition errors ($\beta = -0.07$, $p = 0.046$) (Table 5.2). With respect to gender we found that girls made fewer commission and omission errors than boys ($F(1, 829) = 12.74$, $p < 0.0001$ and $F(1, 829) = 8.00$, $p = 0.005$, respectively) (Table 5.3). A total of 666 children with data on intelligence completed the Auditory Attention task. The results show that IQ is significantly positively associated with overall functioning on this task ($\beta = 0.08$, $p = 0.040$), as well as the number of omission ($\beta = -0.09$, $p = 0.020$) and inhibition errors ($\beta = -0.08$, $p = 0.044$). Children with a higher IQ performed better and made fewer errors (Table 5.4).

A total of 829 children successfully completed the Response Set task. The analyses show that older children had a significantly higher total score than younger children. In addition, older children made fewer commission, omission and inhibition errors (Table 5.2). For all scores of the Response Set task we found a non-linear association with age, again potentially indicating a plateau-effect of performance. For the total score ($\beta = -0.12$, $p < 0.0001$) and the number of commission ($\beta = 0.08$, $p = 0.011$) and omission ($\beta = 0.09$, $p < 0.007$) errors, performance remained relatively stable from the age range 8.5-9 years onwards (Figure 5.3). The number of inhibition errors ($\beta = 0.11$, $p = 0.002$) already remained relatively stable from the age range of 8-8.5 years onwards (Figure 5.3). Regarding gender we found that girls had a significantly higher total score than boys ($F(1, 824) = 16.37$, $p < 0.0001$). Analyses on the amount of commission, omission and inhibition errors also showed that girls made significantly fewer errors compared to boys (all $p < 0.0001$) (Table 5.3). A total of 662 children with data on intelligence successfully completed the Response Set task. The analyses show that children with a higher IQ perform better overall ($\beta = 0.08$, $p = 0.038$) and make fewer commission and omission errors ($\beta = -0.09$, $p = 0.014$ and $\beta = -0.09$, $p = 0.020$, respectively) (Table 5.4).

The Statue task was successfully completed in 187 six year-old children. We found a significant effect of age on the number of sounds made, showing that older children made more sounds ($\beta = 0.15$, $p = 0.036$) (Table 5.2). With respect to gender we found that girls perform significantly better overall than

boys ($F(1,182) = 3.97$, $p = 0.048$) and make fewer movements ($F(1,182) = 4.64$, $p = 0.032$) (Table 5.3). Data on the Statue task was available in 147 six year-old children with IQ data and showed us that children with a higher IQ opened their eyes less frequently during the task ($\beta = -0.18$, $p = 0.045$) (Table 5.4).

Language

Data on the Word Generation task was complete in 803 children. The analysis showed that older children had a better performance on this task than younger children ($\beta = 0.47$, $p < 0.0001$) (Table 5.2). In addition, girls were able to generate significantly more words than boys ($F(1,798) = 4.19$, $p = 0.041$) (Table 5.3). A total of 638 children with data on IQ completed the Word Generation task. The results of the analysis showed that children with a higher IQ were able to generate significantly more words ($\beta = 0.11$, $p = 0.001$) (Table 5.4).

Memory and Learning

A total of 845 children completed the Memory for Faces task. The delayed recall part was completed by 838 children. The results show that older children scored significantly higher on both the immediate and delayed recall ($\beta = 0.23$, $p < 0.0001$ and $\beta = 0.26$, $p < 0.0001$ respectively) (Table 5.2). In addition, we found that girls performed better compared to boys on delayed recall ($F(1, 833) = 4.14$, $p = 0.042$) (Table 5.3). We had complete data on the Memory for Faces task and intelligence in 674 children. The delayed recall part of the task was complete in 668 children with IQ data. We found that performance on the immediate recall part of this task was again positively associated with intelligence ($\beta = 0.10$, $p = 0.007$). No association with intelligence was found for the delayed recall part of the Memory for Faces task (Table 5.4).

The verbal memory task, Narrative Memory, was completed by 652 children and the recognition part of this task was completed by 662 children. Older children had higher scores for the combined free and cued recall score ($\beta = 0.38$, $p < 0.0001$), the free recall only score ($\beta = 0.40$, $p < 0.0001$) and the recognition score ($\beta = 0.18$, $p < 0.0001$) (Table 5.2). With respect to gender differences, we found that girls showed a better performance on the free and cued recall combined ($F(1, 647) = 13.86$, $p < 0.0001$), the free recall only ($F(1, 647) = 14.89$, $p < 0.0001$) and the recognition score ($F(1, 657) = 6.96$, $p = 0.009$) (Table 5.3). A total of 521 children with data on IQ completed the Narrative Memory task. The recognition part of the task was completed by 530 children. The results of the analyses show that children with a higher IQ perform better on both combined free and cued recall, free recall only and recognition ($\beta = 0.16$, $p < 0.0001$, $\beta = 0.14$, $p = 0.001$ and $\beta = 0.16$, $p < 0.0001$, respectively) (Table 5.4).

Sensorimotor Functioning

Complete data on the Visuomotor Precision task was available in 835 children. Evaluating the total time necessary to complete the two items ('Car' and 'Motorcycle'), younger children were slower than older children ($\beta = -0.20$, $p < 0.0001$). We also found a non-linear age association with the amount of errors ($\beta = 0.09$, $p = 0.008$), potentially indicating a plateau-effect in performance with age. Figure 5.3 shows that the number of errors that children make, remains relatively stable from the age range 8-8.5 years onwards. Finally, older children lifted their pencil significantly less than younger children ($\beta = -0.12$, $p = 0.001$) (Table 5.2). With respect to gender we found boys making more errors than girls ($F(1, 830) = 30.26$, $p < 0.0001$) and girls lifting their pencil significantly more often compared to boys ($F(1,$

830) = 9.22, p = 0.002) (Table 5.3). Because some children were extremely quick or slow or made a large amount of errors, we performed additional analyses excluding all children ± 2 SD of the group mean for the total completion time and the amount of errors. Data of these 746 children showed the same findings. Complete data on the Visuomotor Precision task and intelligence was available in 664 children. We found an association between IQ and both the total time needed to complete the task (β = 0.13, p = 0.001) and the number of errors made (β = -0.16, p < 0.0001). A higher IQ was associated with a longer completion time and fewer errors (Table 5.4).

Visuospatial Processing

A total of 840 children completed the Arrows task. Results show that older children performed this task better than younger children (Table 5.2). This association was found to be non-linear (β = -0.09, p = 0.007), potentially indicating a plateau-effect. Performance on this task remained relatively stable from the age range 8.5-9 years of age onwards (Figure 5.3). With respect to gender we found that boys performed better than the girls (F(1, 835) = 31.26, p < 0.0001) (Table 5.3). A total of 670 children with intelligence data completed the Arrows task. Results show that children with a higher IQ perform significantly better (β = 0.16, p < 0.0001) (Table 5.4).

The Geometric Puzzles task was completed by 701 children. Older children had a significantly better performance than younger children on this task (β = 0.35, p < 0.0001) (Table 5.2). No significant differences were found between boys and girls (Table 5.3). Data on the Geometric Puzzles task was complete in 561 children with IQ data. We found a strong positive association between performance on this task and intelligence (β = 0.30, p < 0.0001) (Table 5.4).

A total of 646 children successfully completed the Route Finding task. The results show that older children performed better than younger children (Table 5.2). This age association was non-linear (β = -0.13, p < 0.0001), potentially indicating a plateau-effect of performance with age. Performance on the task remained relatively stable from the age range 8-8.5 years onwards (Figure 5.3). Furthermore, boys had a higher total score than girls (F(1, 641) = 6.08, p = 0.014) (Table 5.3). Finally, the Route Finding task was successfully collected in 519 children with IQ data. Again, the analysis shows that children with a higher IQ perform significantly better on this task (β = 0.27, p < 0.0001) (Table 5.4).

TABLE 5.2. **THE ASSOCIATION OF AGE WITH NEPSY-II-NL TASK PERFORMANCE**

TASK	n	AGE[a]	AGE SQUARED[a]
ATTENTION AND EXECUTIVE FUNCTIONING			
Auditory Attention			
• Total score[b]	834	$\beta = 0.18$, $p < 0.0001$	n.s.
• Commission errors[b]	834	$\beta = -0.24$, $p < 0.0001$	$\beta = 0.08$, $p = 0.026$
• Omission errors[b]	834	$\beta = -0.34$, $p < 0.0001$	n.s.
• Inhibition errors[b]	834	$\beta = -0.07$, $p = 0.046$	n.s.
Response Set			
• Total score[b]	829	$\beta = 0.37$, $p < 0.0001$	$\beta = -0.12$, $p < 0.0001$
• Commission errors[b]	829	$\beta = -0.34$, $p < 0.0001$	$\beta = 0.08$, $p = 0.011$
• Omission errors[b]	829	$\beta = -0.38$, $p < 0.0001$	$\beta = 0.09$, $p = 0.007$
• Inhibition errors[b]	829	$\beta = -0.30$, $p < 0.0001$	$\beta = 0.11$, $p = 0.002$
Statue[c,d]			
• Total score[b]	187	n.s.	n.s.
• Total movements[b]	187	n.s.	n.s.
• Total sounds[b]	187	$\beta = 0.15$, $p = 0.036$	n.s.
• Total eye openings[b]	187	n.s.	n.s.
LANGUAGE			
Word Generation			
• Total semantic score	803	$\beta = 0.47$, $p < 0.0001$	n.s.
MEMORY AND LEARNING			
Memory for Faces			
• Total score	845	$\beta = 0.23$, $p < 0.0001$	n.s.
Memory for Faces – delayed			
• Total score	838	$\beta = 0.26$, $p < 0.0001$	n.s.
Narrative Memory[c]			
• Total score free and cued recall	652	$\beta = 0.38$, $p < 0.0001$	n.s.
• Total score free recall	652	$\beta = 0.40$, $p < 0.0001$	n.s.
• Total score recognition[b]	662	$\beta = 0.18$, $p < 0.0001$	n.s.
SENSORIMOTOR FUNCTION			
Visuomotor Precision			
• Total time[b]	835	$\beta = -0.20$, $p < 0.0001$	n.s.
• Total errors[b]	835	$\beta = -0.27$, $p < 0.0001$	$\beta = 0.09$, $p = 0.008$
• Total pencil lifts[b]	835	$\beta = -0.12$, $p = 0.001$	n.s.
VISUOSPATIAL PROCESSING			
Arrows			
• Total score[b]	840	$\beta = 0.36$, $p < 0.0001$	$\beta = -0.09$, $p = 0.007$
Geometric Puzzles[c]			
• Total score	701	$\beta = 0.35$, $p < 0.0001$	n.s.
Route Finding[c]			
• Total score[b]	646	$\beta = 0.36$, $p < 0.0001$	$\beta = -0.13$, $p < 0.0001$

NOTE. Regression analyses were performed. All analyses are adjusted for child ethnicity and gender.
[a] In case of the presence of a non-linear age association, effect estimates (linear and quadratic) of the quadratic model are provided. If there was no non-linear effect, the effect estimate of the linear model is provided. [b] Mathematically transformed score was used. [c] Not assessed in the shortened NEPSY-II-NL battery that was administered in a subgroup of the participants. [d] Analyses performed only in 6 year-old children.

TABLE 5.3. **THE ASSOCIATION OF GENDER WITH NEPSY-II-NL TASK PERFORMANCE**

TASK	n	GENDER
ATTENTION AND EXECUTIVE FUNCTIONING		
Auditory Attention		
• Total score[a]	834	n.s.
• Commission errors[a]	834	$F_{1,829} = 12.74$, $p < 0.0001$
• Omission errors[a]	834	$F_{1,829} = 8.00$, $p = 0.005$
• Inhibition errors[a]	834	n.s.
Response Set		
• Total score[a]	829	$F_{1,824} = 16.37$, $p < 0.0001$
• Commission errors[a]	829	$F_{1,824} = 30.39$, $p < 0.0001$
• Omission errors[a]	829	$F_{1,824} = 22.60$, $p < 0.0001$
• Inhibition errors[a]	829	$F_{1,824} = 12.38$, $p < 0.0001$
Statue[b,c]		
• Total score[a]	187	$F_{1,182} = 3.97$, $p = 0.048$
• Total movements[a]	187	$F_{1,182} = 4.64$, $p = 0.032$
• Total sounds[a]	187	n.s.
• Total eye openings[a]	187	n.s.
LANGUAGE		
Word Generation		
• Total semantic score	803	$F_{1,798} = 4.19$, $p = 0.041$
MEMORY AND LEARNING		
Memory for Faces		
• Total score	845	n.s.
Memory for Faces – delayed		
• Total score	838	$F_{1,833} = 4.14$, $p = 0.042$
Narrative Memory[b]		
• Total score free and cued recall	652	$F_{1,647} = 13.86$, $p < 0.0001$
• Total score free recall	652	$F_{1,647} = 14.89$, $p < 0.0001$
• Total score recognition[a]	662	$F_{1,657} = 6.96$, $p = 0.009$
SENSORIMOTOR FUNCTION		
Visuomotor Precision		
• Total time[a]	835	n.s.
• Total errors[a]	835	$F_{1,830} = 30.26$, $p < 0.0001$
• Total pencil lifts[a]	835	$F_{1,830} = 9.22$, $p = 0.002$
VISUOSPATIAL PROCESSING		
Arrows		
• Total score[a]	840	$F_{1,835} = 31.26$, $p < 0.0001$
Geometric Puzzles[b]		
• Total score	701	n.s.
Route Finding[b]		
• Total score[a]	646	$F_{1,641} = 6.08$, $p = 0.014$

NOTE. ANOVA was used. All analyses are adjusted for child ethnicity and age.

[a] Mathematically transformed score was used. [b] Not assessed in the shortened NEPSY-II-NL battery that was administered in a subgroup of the participants. [c] Analyses performed only in 6 year-old children.

TABLE 5.4. **THE ASSOCIATION OF INTELLIGENCE WITH NEPSY-II-NL TASK PERFORMANCE**

TASK	n	INTELLIGENCE
ATTENTION AND EXECUTIVE FUNCTIONING		
Auditory Attention		
• Total score[a]	666	β = 0.08, p = 0.040
• Commission errors[a]	666	n.s.
• Omission errors[a]	666	β = -0.09, p = 0.020
• Inhibition errors[a]	666	β = -0.08, p = 0.044
Response Set		
• Total score[a]	662	β = 0.08, p = 0.038
• Commission errors[a]	662	β = -0.09, p = 0.014
• Omission errors[a]	662	β = -0.09, p = 0.020
• Inhibition errors[a]	662	n.s.
Statue[b,c]		
• Total score[a]	147	n.s.
• Total movements[a]	147	n.s.
• Total sounds[a]	147	n.s.
• Total eye openings[a]	147	β = -0.18, p = 0.045
LANGUAGE		
Word Generation		
• Total semantic score	638	β = 0.11, p = 0.001
MEMORY AND LEARNING		
Memory for Faces		
• Total score	674	β = 0.10, p = 0.007
Memory for Faces – delayed		
• Total score	668	n.s.
Narrative Memory[b]		
• Total score free and cued recall	521	β = 0.16, p < 0.0001
• Total score free recall	521	β = 0.14, p = 0.001
• Total score recognition[a]	530	β = 0.16, p < 0.0001
SENSORIMOTOR FUNCTION		
Visuomotor Precision		
• Total time[a]	664	β = 0.13, p = 0.001
• Total errors[a]	664	β = -0.16, p < 0.0001
• Total pencil lifts[a]	664	n.s.
VISUOSPATIAL PROCESSING		
Arrows		
• Total score[a]	670	β = 0.16, p < 0.0001
Geometric Puzzles[b]		
• Total score	561	β = 0.30, p < 0.0001
Route Finding[b]		
• Total score[a]	519	β = 0.27, p < 0.0001

NOTE. Regression analyses were performed. All analyses are adjusted for child age, gender and ethnicity. Total n with IQ data = 679.
[a] Mathematically transformed score was used. [b] Not assessed in the shortened NEPSY-II-NL battery that was administered in a subgroup of the participants. [c] Analyses performed only in 6 year-old children.

TABLE 5.5. **PARTIAL CORRELATIONS OF INTELLIGENCE WITH NEPSY-II-NL TASK PERFORMANCE**

TASK	n	PARTIAL CORRELATION WITH INTELLIGENCE
ATTENTION AND EXECUTIVE FUNCTIONING		
Auditory Attention		
• Total score	666	r = 0.10, p = 0.013
• Commission errors	666	n.s.
• Omission errors	666	r = -0.10, p = 0.012
• Inhibition errors	666	n.s.
Response Set		
• Total score	662	r = 0.09, p = 0.023
• Commission errors	662	r = -0.09, p = 0.020
• Omission errors	662	r = -0.09, p = 0.023
• Inhibition errors	662	n.s.
Statue[a,b]		
• Total score	147	n.s.
• Total movements	147	n.s.
• Total sounds	147	n.s.
• Total eye openings	147	n.s.
LANGUAGE		
Word Generation		
• Total semantic score	638	r = 0.13, p = 0.001
MEMORY AND LEARNING		
Memory for Faces		
• Total score	674	r = 0.10, p = 0.007
Memory for Faces – delayed		
• Total score	668	n.s.
Narrative Memory[a]		
• Total score free and cued recall	521	r = 0.18, p < 0.0001
• Total score free recall	521	r = 0.15, p < 0.0001
• Total score recognition	530	r = 0.15, p = 0.001
SENSORIMOTOR FUNCTION		
Visuomotor Precision		
• Total time	664	r = 0.13, p = 0.001
• Total errors	664	r = -0.17, p < 0.0001
• Total pencil lifts	664	n.s.
VISUOSPATIAL PROCESSING		
Arrows		
• Total score	670	r = 0.20, p < 0.0001
Geometric Puzzles[a]		
• Total score	561	r = 0.32, p < 0.0001
Route Finding[a]		
• Total score	519	r = 0.35, p < 0.0001

NOTE. Partial correlations adjusted for child age, gender and ethnicity are shown. Total n with IQ data = 679.

[a] Not assessed in the shortened NEPSY-II-NL battery that was administered in a subgroup of the participants. [b] Analyses performed only in 6 year-old children.

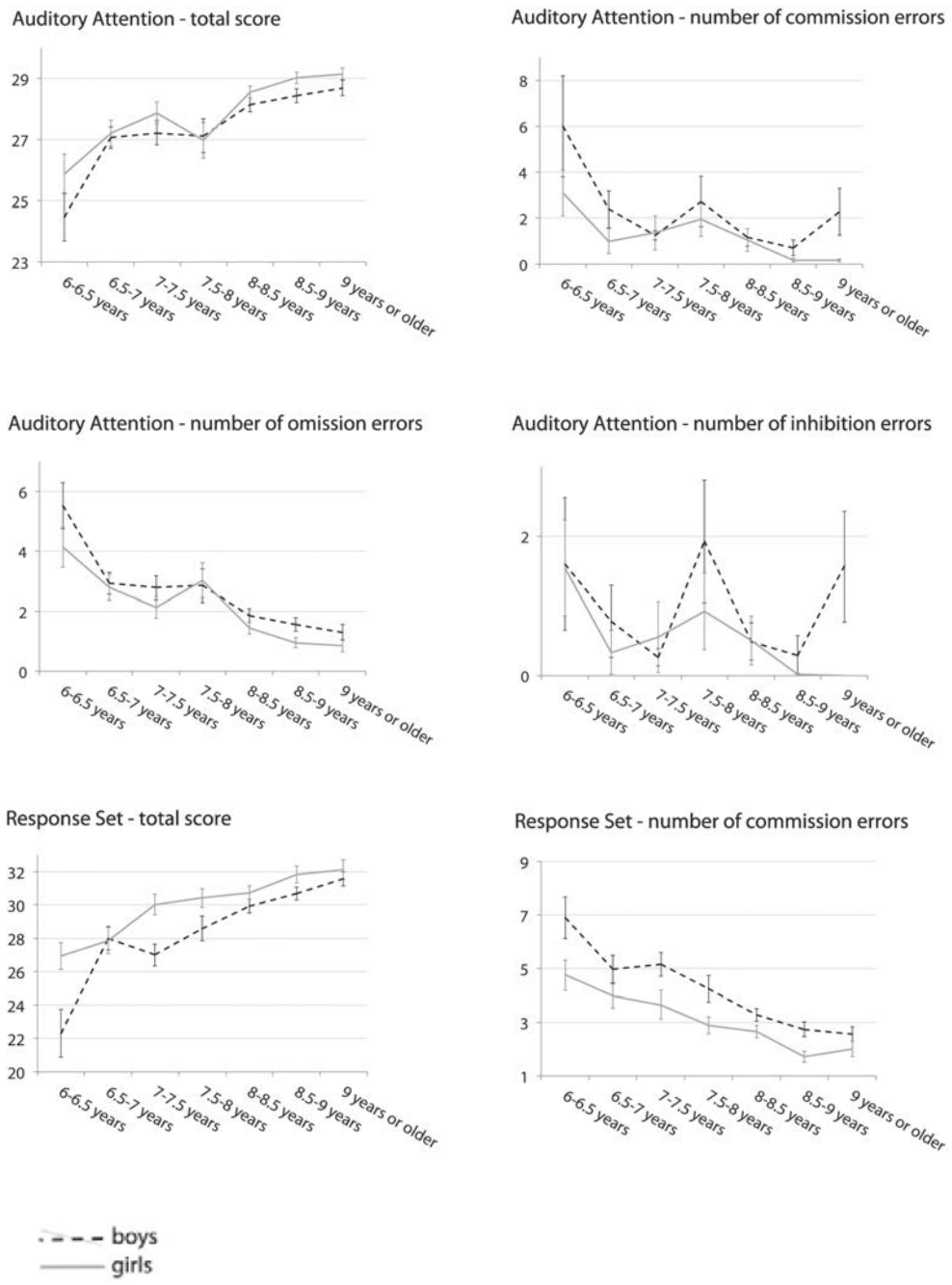

FIGURE 5.2. Gender- and Age-related Trajectories in NEPSY-II-NL.
(Unadjusted) mean scores and standard errors are presented. The exact number of children per age category depicted differs per task, but proportions were roughly 9% (6-6.5), 13% (6.5-7), 13% (7-7.5), 15% (7.5-8), 24% (8-8.5), 15% (8.5-9) and 11% (9 and older).

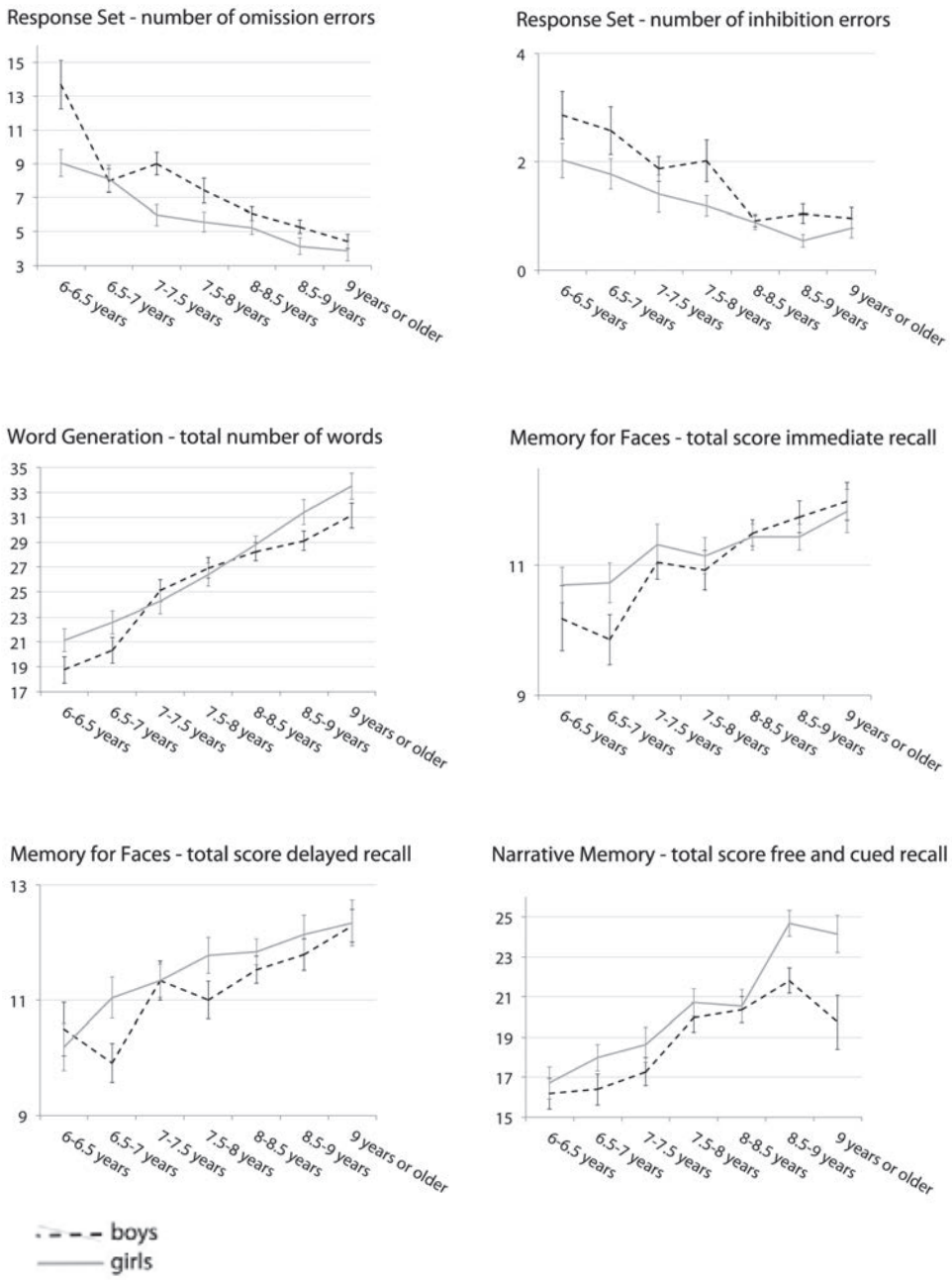

FIGURE 5.2. Gender- and Age-related Trajectories in NEPSY-II-NL. (continued)
(Unadjusted) mean scores and standard errors are presented. The exact number of children per age category depicted differs per task, but proportions were roughly 9% (6-6.5), 13% (6.5-7), 13% (7-7.5), 15% (7.5-8), 24% (8-8.5), 15% (8.5-9) and 11% (9 and older).

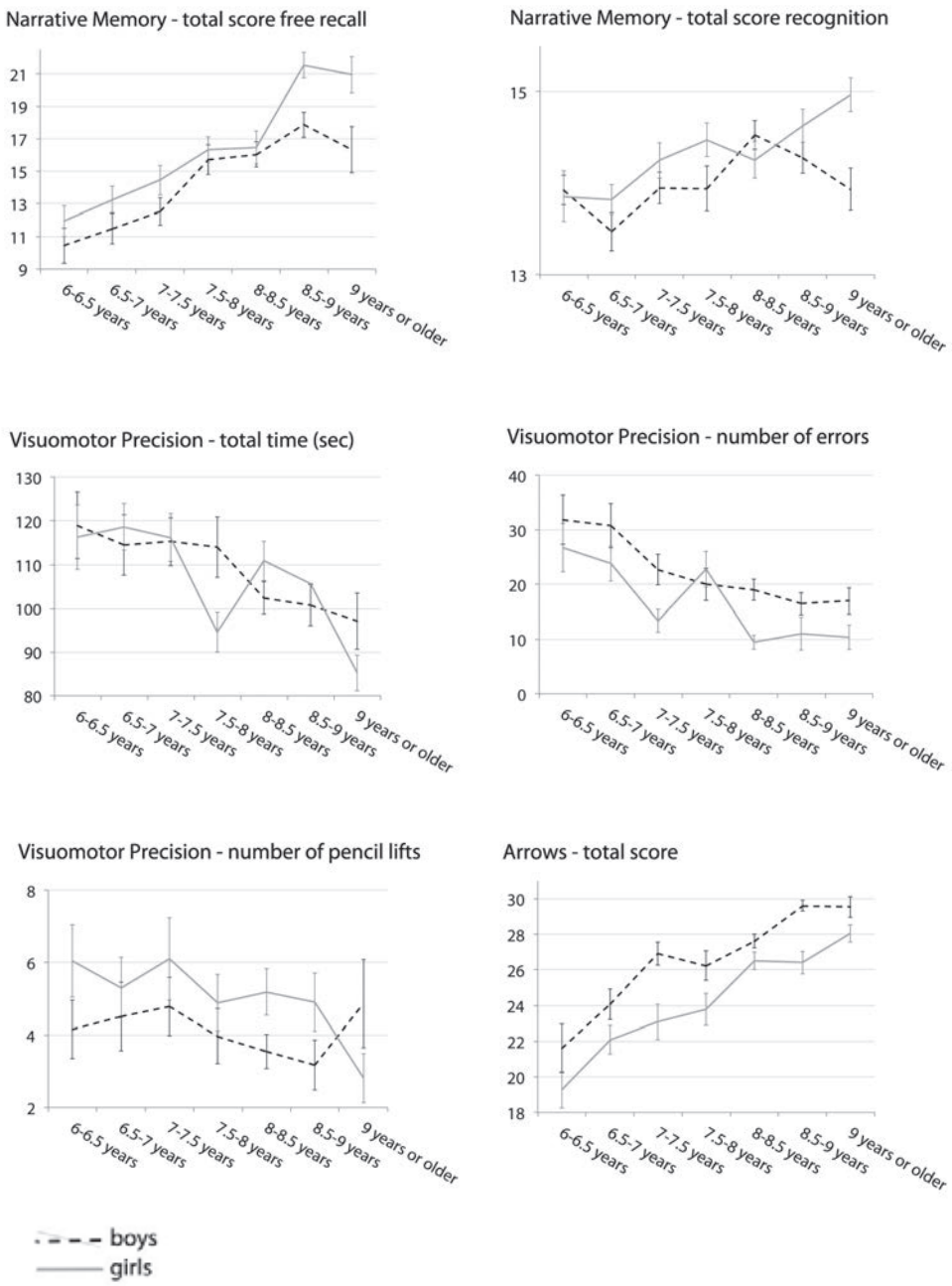

FIGURE 5.2. Gender- and Age-related Trajectories in NEPSY-II-NL. (continued)
(Unadjusted) mean scores and standard errors are presented. The exact number of children per age category depicted differs per task, but proportions were roughly 9% (6-6.5), 13% (6.5-7), 13% (7-7.5), 15% (7.5-8), 24% (8-8.5), 15% (8.5-9) and 11% (9 and older)

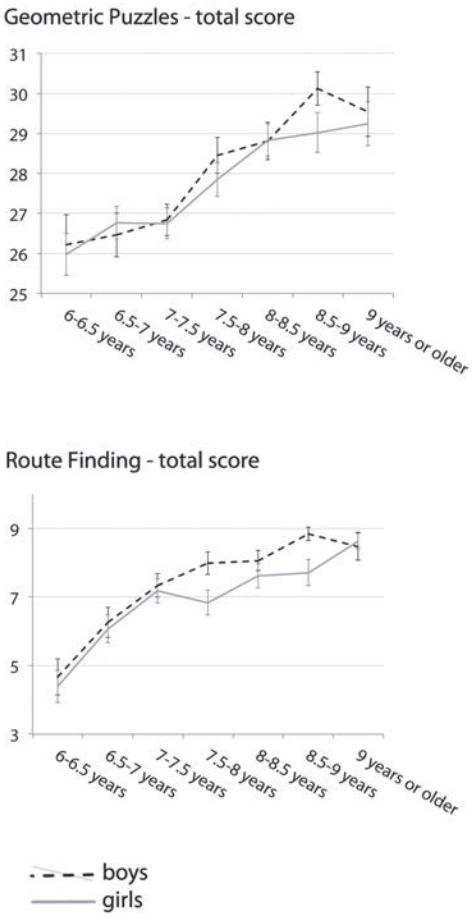

FIGURE 5.2. Gender- and Age-related Trajectories in NEPSY-II-NL (continued).
(Unadjusted) mean scores and standard errors are presented. The exact number of children per age category depicted differs per task, but proportions were roughly 9% (6-6.5), 13% (6.5-7), 13% (7-7.5), 15% (7.5-8), 24% (8-8.5), 15% (8.5-9) and 11% (9 and older).

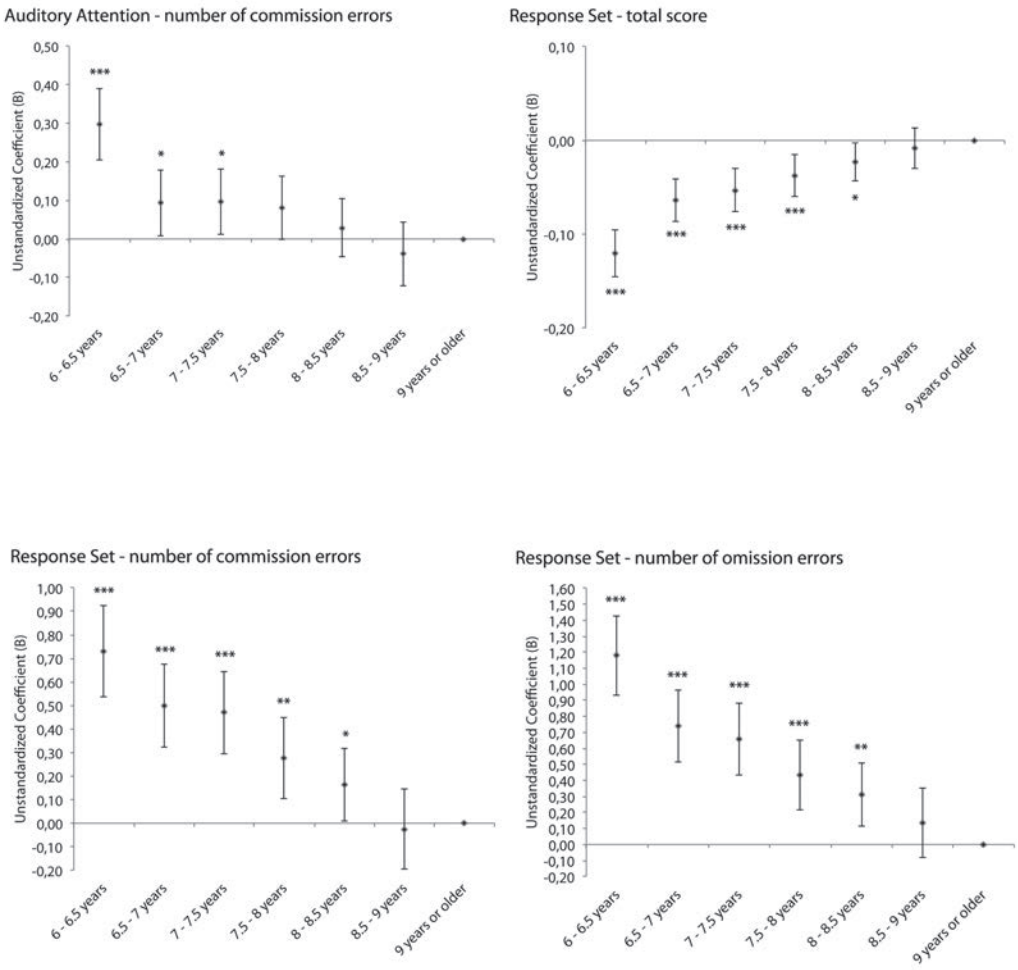

FIGURE 5.3. Illustration of non-linear age associations.
Presented are unstandardized regression coefficients (B's) and confidence intervals, oldest age group used as reference category. Analyses adjusted for child gender and ethnicity. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

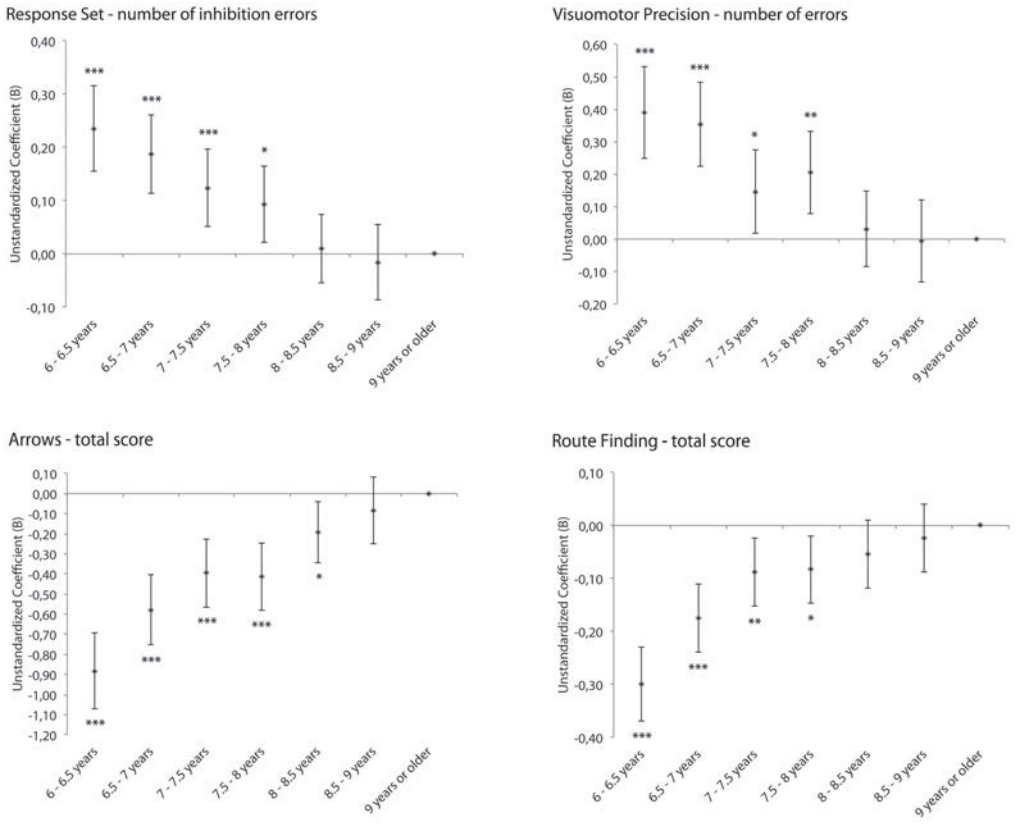

FIGURE 5.3. Illustration of non-linear age associations. (continued)
Presented are unstandardized regression coefficients (B's) and confidence intervals, oldest age group used as reference category. Analyses adjusted for child gender and ethnicity. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

DISCUSSION

In this study, we performed an extensive neuropsychological assessment in a large group (n=853) of young typically developing children, using the NEPSY-II-NL (Brooks et al., 2010). Different domains of neuropsychological development were assessed, i.e. attention and executive functioning, language, memory, sensorimotor functioning and visuospatial processing. Associations of gender, age, and intelligence with performance were studied.

First, our results clearly show an effect of gender on performance for the majority of the assessed tasks in this age range. In most tasks, girls performed better compared to boys. However, as hypothesized, there were two tasks in which boys outperformed the girls, i.e. Arrows and Route Finding, which are both part of the visuospatial processing domain. Previous research has shown that boys tend to

perform better than girls on tasks requiring visuospatial abilities (like visuospatial perception and orientation) (Linn & Petersen, 1985; Voyer, 2011). The basis of this gender difference in visuospatial abilities is unclear. It may be due to neurobiological differences, such as differences in white matter development between boys and girls (De Bellis et al., 2001), but may also be attributable to different experiences of boys and girls that are important for the acquisition, selection and use of strategies in visuospatial processing (Linn & Petersen, 1985). Our hypothesized gender difference in favor of girls on language tasks was also supported; in the Word Generation task we found that girls were able to generate significantly more words compared to boys.

Interestingly, we are the first to assess gender differences on neuropsychological functioning measured with the NEPSY testbattery. Previous studies on both the original NEPSY (Korkman et al., 2001) and the NEPSY-II (Brooks et al., 2010; Korkman et al., 2013) did not address gender differences. In addition, the NEPSY-II norms do not discriminate between boys and girls (Korkman et al., 2010b). Based on our findings and the knowledge that boys and girls differ in their (neuro)cognitive development, gender differences should be taken into account when using the NEPSY-II-NL in the clinical practice or for research purposes. It might even be advisable to create separate norms for boys and girls.

Not unexpected, we found that in the majority of the tasks performance was age-dependent, in a sense that older children perform better than younger children. Even though our study sample covered a small age range, considerable age-related differences were evident. This is in line with previous studies showing the early school-age period of a child's life to be a period of rapid neurocognitive development (Casey et al., 2005; Giedd et al., 1999; Gogtay et al., 2004). It is also in line with previous studies on the NEPSY(-II) (Korkman et al., 2001; Korkman et al., 2013) that showed that age effects were most pronounced between 5 and 10 years of age.

By repeating the analyses with a quadratic age-term included in the model, we were able to examine potential non-linear age effects, that might indicate a plateau-effect of performance with age. For a number of tasks (the number of commission errors of the Auditory Attention task, Response Set, the number of errors of the Visuomotor Precision task, Arrows and Route Finding) we did find a non-linear association with age. For these tasks we found that performance remained relatively stable from a certain age range onwards. Furthermore, the analyses show that development seems to go fastest in the youngest children in some of the tasks. Our hypothesis that simple (visuo)motor functions will be mastered in the age range of our sample is partly supported by our data since the amount of errors of the Visuomotor Precision task remains stable from the age range 7.5-8 years onwards. However, the time needed to complete the task and the number of pencil lifts, which are also measures of motor development, did not reach a plateau, suggesting continued development. We also found two visuospatial tasks (Arrows and Route Finding) to show a non-linear age-effect in the age range of our study. Previous studies have shown that visuospatial abilities appear to only reach mastery around the beginning of adolescence (Del Giudice et al., 2000; Korkman et al., 2013; Rosselli et al., 2010). And indeed, when looking at Figure 5.3, it seems that (although the older age groups did not differ significantly from the oldest age group) performance in these tasks is still increasing, but at a slower rate. This might mean that peak performance has not been reached yet.

The only scores in our study in which the influence of age was not apparent, were most of the Statue task scores. However, this is likely because of the small age range for which this task is suitable, and one would not expect a large amount of development in such a small age range. The non-significant

findings may also be attributable to the relatively small sample, providing not enough power to detect such small differences.

One of the most complex tasks of our test battery in terms of interpretation was the Visuomotor Precision task. Due to the speed/performance trade off the analyses were less straightforward. During the assessment we noticed that the choice of strategy differs between children, since some children tried to be as fast as possible (and paid less attention to the amount of errors), while other children tried to make as few mistakes as possible (and paid less attention to their speed). We did not find any gender related differences for the amount of time it took to complete the two items, but we did find that girls were more accurate during the task since they made fewer errors, although they lifted their pencil more often than boys. As expected, older children were faster, more accurate and lifted their pencil less often than younger children, indicating that their visuomotor abilities are more developed. Even after excluding children ± 2 SD of the group mean for total completion time and number of errors, the results remained similar. In the NEPSY-II-NL manual, the amount of errors made during the Visuomotor Precision task and the total time are separate scores, no combined score exists. However, we suggest that the speed/accuracy trade off requires a joint interpretation. In Figure 5.4 we present a scatterplot showing both the number of errors made and the total time needed. This figure was made in a smaller sample (n=746) in which outliers (± 2 SD from group mean) on the total completion time and number of errors were excluded. As expected the figure shows that, even after excluding the extremes, children that are faster tend to make more errors, while children that are slower generally make fewer errors. It also clearly shows that the speed/performance trade off improves (faster and fewer errors) with increasing age.

With respect to intelligence we found that performance on nearly all tasks showed an association with nonverbal IQ, indicating that children with a higher IQ performed significantly better. The fact that the IQ measure was obtained on average 1.7 years earlier could be regarded as a limitation. However, the found relationship between neuropsychological performance and earlier measured IQ in a way also reflects a level of stability in cognitive functioning. The only tasks in which performance did not show a significant association with intelligence were the Auditory Attention number of commission errors, Response Set number of inhibition errors, most of the scores of the Statue task, the delayed recall score of the Memory for Faces task and the number of pencil lifts of the Visuomotor Precision task. Partial correlations show that performance on tasks of the Visuospatial Processing domain have the strongest association with intelligence, with correlation coefficients ranging between 0.20 and 0.35. This might partly be explained the nonverbal nature of both the visuospatial tasks and the IQ test that was used, although some other NEPSY-II-NL tasks that are also expressively nonverbal (such as the Auditory Attention, Response Set, Statue, Memory for Faces and the Visuomotor Precision task) show weaker correlations with IQ. As hypothesized, we found performance in the domain Attention and Executive Functioning to be least strongly correlated with intelligence. Of the Auditory Attention and Response Set tasks, the number of commission and inhibition errors showed the weakest (and mostly non-significant) correlations, which is interesting since both scores represent response inhibition (executive functioning) more than just the ability to pay attention. Although most of the tasks show a clear association with intelligence, we do not necessarily conclude that one should control for intelligence when assessing neuropsychological functioning. As Dennis et al. (2009) have pointed out, controlling for IQ in cognitive studies of neurodevelopmental disorders (such as attention deficit/hyperactivity disorder) might even remove some of the true variance, hindering a proper interpretation of findings

(Dennis et al., 2009). However, in some cases controlling for IQ might be advisable, for example when one would want to study specific problems that are not explained by general intelligence.

Strengths of the current study include the large sample size and the narrow age range of the children. Since the first (school)years of the child's life is a period of rapid neurocognitive development (Casey et al., 2005; Giedd et al., 1999; Gogtay et al., 2004), it is very important to examine children's cognitive abilities during this age range. Understanding typical development will also help us to better understand aberrant (cognitive) development in young children. A limitation is the cross-sectional character of our study. Since we have not performed longitudinal assessment, we are not able to evaluate true age-related trajectories. In addition, because of time constraints, we were unfortunately not able to administer the entire NEPSY-II-NL battery of 34 tasks.

To conclude, in the current study in 853 typically developing children between 6 and 10 years of age, we found clear gender-, age- and intelligence related differences on various tasks assessing the neuropsychological domains attention, executive functioning, language, memory, sensorimotor functioning and visuospatial processing. In nearly all tasks, older children performed better. In addition to age, performance on the majority of the assessed NEPSY-II-NL tasks was also related to intelligence, although not all neuropsychological domains showed an equally strong association with intelligence. With respect to gender differences we found that in the majority of the tasks girls outperformed boys, with the exception of two tasks that require visuospatial abilities, in which boys performed better than girls. Since, gender differences in performance on the NEPSY(-II) have not been previously described and are not being taken into account when calculating normative scores, this study argues for the development of separate normative scores for boys and girls.

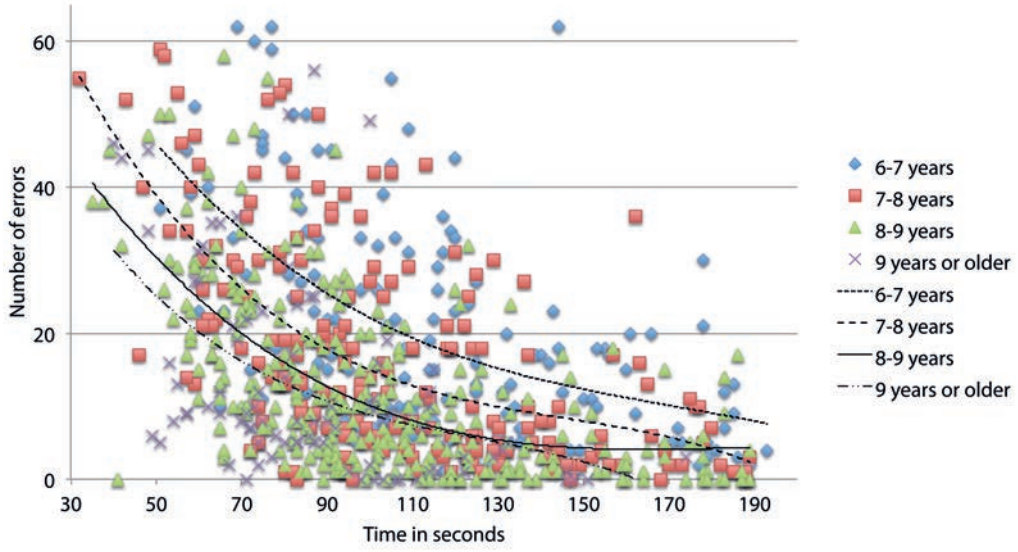

FIGURE 5.4. Scatterplot of number of errors and time for Visuomotor Precision task.
Reduced n=746 (outliers +/- 2sd from mean were excluded). Fit lines are polynomials.

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CHAPTER

6

White matter integrity and cognitive performance in school-age children

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Neuroimage, 2015, epub ahead of print

ABSTRACT

Background

Child and adolescent brain development are typically accompanied by marked improvements in a wide range of cognitive abilities. However, limited information is available surrounding the role of white matter in shaping cognitive abilities in children.

Methods

The current study examined associations between white matter microstructure and cognitive performance in a large sample (n = 778) of 6-to-10 year-old children.

Results

Results show white matter microstructure is related to non-verbal intelligence and to visuospatial ability, independent of age. Specificity was demonstrated, as white matter associations with visuospatial ability were independent of general intellectual ability. Associations between white matter integrity and cognition were similar in boys and girls.

Conclusions

In summary, results demonstrate white matter structure-function associations are present in children, independent of age and broader cognitive abilities. The presence of such associations in the general population is informative for studies examining child psychopathology.

INTRODUCTION

Magnetic resonance imaging (MRI) studies demonstrate significant neurodevelopmental changes throughout childhood and adolescence, into young-adulthood. These neurodevelopmental changes occur concurrently with observed improvements in a wide range of cognitive abilities. White matter development, including myelination, continues throughout childhood and adolescence and is thought to play a key role in cognitive function. As distant brain regions become more efficiently interconnected, it is expected that the ability to utilize and manipulate information also becomes more efficient. The role of white matter in shaping cognitive abilities has been previously explored, however the literature in children, especially studies with large sample sizes, remains limited. Further, while such structure-function associations seem intuitive, current in vivo neurobiological measures of the brain do not always demonstrate a straightforward link with neuropsychological performance, especially in the absence of severe neurological or psychiatric symptoms.

White matter maturational effects have been studied in vivo for over a decade using morphological information (i.e., volume, density) and, more recently, using measures of microstructural integrity (Lenroot and Giedd, 2006; Schmithorst and Yuan, 2010). Diffusion tensor imaging (DTI) is a non-invasive technique that provides such microstructural information related to white matter status (Basser et al., 1994). White matter integrity is inferred from DTI based on its ability to measure patterns of water diffusion in the brain. The water diffusion profile in white matter is distinct from that of gray matter due to the myelin sheath, axonal arrangement and packing, and axonal diameter (Beaulieu, 2002). Common parameters describing white matter integrity from DTI include fractional anisotropy (FA) and mean diffusivity (MD).

Beginning with morphological information from structural imaging, and more recently with DTI, white matter development in children and adolescents has been examined using both cross-sectional and longitudinal designs (Barnea-Goraly et al., 2005; Giedd et al., 1999; Giorgio et al., 2008; Lebel et al., 2008; Schmithorst et al., 2002; Schmithorst and Yuan, 2010). The majority of literature in children demonstrates that with age, both white matter volume and microstructural integrity increase. The precise determinant of these maturational effects has yet to be fully delineated, however the primary hypothesis suggests a combination of increases in myelination coupled with an optimized structural organization of axons (Paus, 2010). Interestingly, studies have demonstrated differential developmental trajectories in white matter between boys and girls (Erus et al., 2015; Simmonds et al., 2014), which may underlie some of the subtle cognitive differences (Maitland et al., 2000).

While studied to a lesser extent than white matter maturation, associations between white matter and cognitive performance have also been examined in children (Erus et al., 2015; Fryer et al., 2008; Johansen-Berg et al., 2007; Muetzel et al., 2008; Navas-Sanchez et al., 2014; Schmithorst et al., 2005). In an early study of roughly 50 children, 5-to-18 years old, Schmithorst et al. (2005) found positive associations between white matter microstructure (i.e., DTI metrics) and intelligence, irrespective of age and sex. A more recent study of 36 children and adolescents 11-to-15 years of age also showed a positive association between white matter microstructure and intelligence (Navas-Sanchez et al., 2014). In general, available studies of white matter microstructure and cognitive ability demonstrate brain-behavior associations that suggest white matter integrity is linked to better cognitive performance.

The current study aims to describe associations between white matter microstructure and cognition

across a wide range of neuropsychological domains in a large sample of 6-to-10 year-old children. We hypothesized age-independent, positive associations between FA and cognitive performance across all domains. As previous work has already demonstrated distinct patterns of white matter maturation in boys and girls, we also hypothesize differential structure-function associations in boys and girls, specifically in cognitive domains where differences in ability have been demonstrated (e.g., language and spatial ability).

MATERIALS AND METHODS

Participants

The current study is embedded within the Generation R Study, which is a large, population-based cohort investigating children's health from fetal life onwards in Rotterdam, the Netherlands (Jaddoe et al., 2012). A sub-sample of 1,070 children visited the research center for neuropsychological testing and MRI scanning. Further details of the selection and recruitment of subjects, the research protocol, and overall design of this MRI sub-study are described elsewhere (White et al., 2013). Of the 1,070 children who visited the research center for an MRI, 1,033 received a DTI scan. Of the 1,033 DTI scans, 255 (25%) were excluded due to excessive motion / artifact (described below), leaving 778 datasets for analysis. The Medical Ethics Committee of the Erasmus Medical Center approved all study procedures, and parents provided written informed consent.

Intelligence Assessment

General intellectual functioning was assessed during the age-6 assessment wave using an abbreviated version of the Snijders-Oomen Niet-verbale Intelligentie Test – Revisie (SON-R 2½-7) (Tellegen et al., 2005; Tiemeier et al., 2012). The SON-R 2½-7 is a measure of non-verbal intelligence for children between 2.5 and 7 years of age and was selected in order to minimize language-dependent confounds that may be present in a large, ethnically diverse sample such as the Generation R Study. An intelligence quotient (IQ) was estimated from the two SON-R performance subtests that were administered (Mosaics and Categories), which is highly correlated with estimates resulting from the complete version (Basten et al., 2014).

Neuropsychological Assessment

Neuropsychological functioning was assessed using the NEPSY-II-NL, a Dutch translation and adaptation of the NEPSY-II (Brooks et al., 2010). A selection of tests from the NEPSY was chosen in order to examine five areas of cognitive ability: attention and executive functioning, language, memory and learning, sensorimotor functioning, and visuospatial processing (White et al., 2013). In order to limit the number of statistical tests performed, and because the NEPSY-II-NL does not provide domain-specific summary scores, a data reduction technique was utilized to derive empirical scores. An overall total performance score was derived by using a principal component analysis (PCA) on all test scores from the NEPSY-II-NL and selecting the first unrotated factor score. Next, for each of the five cognitive abilities, NEPSY-II-NL test items belonging to a given domain were submitted to PCA, and again the first unrotated factor score was selected as the summary score for that cognitive domain.

Assessment of Behavioral Problems

Behavioral problems in children were assessed using the Child Behavior Checklist (CBCL/1½-5) from the age-6 assessment wave (Tiemeier et al., 2012). All children were assessed with one instrument; the preschool CBCL was selected because many children were younger than six years of age at the time of the assessment, and the other versions are inappropriate for such young children. The CBCL is a 99-item parental report inventory that utilizes a Likert response format ("Not True", "Somewhat True", "Very True") for a variety of behaviors. A simple sum of all items was used to create a total behavioral problems score, which was square root transformed to approximate a normal distribution (Achenbach and Rescorla, 2000).

MR-Image Acquisition

Prior to neuroimaging, all children underwent a 30-minute mock scanning session in order to acclimate them to the MR-environment (White et al., 2013). Magnetic resonance imaging data were acquired on a 3 Tesla GE MR-750 system (General Electric, Milwaukee, WI). A short, three-plane localizer sequence was initially run and used to position all subsequent scans. Diffusion tensor imaging data were acquired using a single-shot, echo-planar imaging sequence with the following parameters: TR = 11,000 ms, TE = 83 ms , flip angle = 90, matrix = 128 x 128, FOV = 256 mm x 256 mm, slice thickness = 2 mm, number of slices = 77, acquisition time = 7 min 40 sec. In total, 35 volumes with diffusion weighing (b = 1000 s/mm^2) and 3 volumes without diffusion weighting (b = 0 s/mm^2) were acquired.

MR-Image Preprocessing

Data were processed using the Functional MRI of the Brain's Software Library (FMRIB, FSL, Jenkinson et al., 2012) and the Camino Diffusion MRI Toolkit (Cook et al., 2006). Image processing tools were executed in Python (version 2.7) through the Neuroimaging in Python Pipelines and Interfaces package (Nipype, version 0.92) (Gorgolewski et al., 2011). Images were first adjusted for motion and eddy-current induced artifacts (Haselgrove and Moore, 1996) using the FSL "eddy_correct" tool (Jenkinson and Smith, 2001). The resulting transformation matrices were then used to rotate the gradient direction table, in order to account for the rotations applied to the image data (Jones and Cercignani, 2010; Leemans and Jones, 2009). Non-brain tissue was removed using the FSL Brain Extraction Tool (Smith, 2002). As limitations have been cited with respect to the ordinary least squares method (Veraart et al., 2013), the diffusion tensor was fit using the RESTORE method implemented in Camino (Chang et al., 2005), and common scalar maps (i.e., FA, MD, axial diffusivity (AD), radial diffusivity (RD)) were then computed.

Probabilistic Fiber Tractography

Fully automated probabilistic fiber tractography was performed using the FSL plugin, "AutoPtx" (de Groot et al., in press). The method generates subject-specific, probabilistic representations of multiple white matter fiber bundles using FSL tools. Briefly, the diffusion data were first processed using the Bayesian Estimation of Diffusion Parameters Obtained using Sampling Techniques (BEDPOSTx), accounting for two fiber orientations at each voxel (Behrens et al., 2007; Behrens et al., 2003). Next, for each subject, the FA map was aligned to the FMRIB-58 FA template image with the FSL nonlinear registration tool (FNIRT). The inverse of this nonlinear warp field was computed, and applied to a series

of predefined seed, target, exclusion, and termination masks provided by the AutoPtx plugin (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/AutoPtx). Probabilistic fiber tracking was then performed with the FSL Probtrackx module using these supplied tract-specific masks (i.e., seed, target, etc.) that were warped to the native diffusion image space of each subject (Behrens et al., 2007). The resulting path distributions were normalized to a scale from 0 to 1 using the total number of successful seed-to-target attempts, and were subsequently thresholded to remove low-probability voxels likely related to noise. For each tract, the number of samples used for probabilistic tracking, and the probability thresholds applied to the resulting distributions, were selected based on previously established values (de Groot et al., in press). After the tracts were thresholded, average DTI scalar measures were computed within each tract. The tracts used in the current analyses are presented in Table 6.1.

TABLE 6.1. **CONFIRMATORY FACTOR ANALYSIS OF FA IN WHITE MATTER TRACTS**

TRACT	HEMISPHERE	STANDARDIZED FACTOR LOADING
Cingulum Bundle	Left	0.640
	Right	0.619
Corticospinal Tract	Left	0.386
	Right	0.392
Forceps Major	-	0.614
Forceps Minor	-	0.382
Inferior Longitudinal Fasciculus	Left	0.733
	Right	0.733
Superior Longitudinal Fasciculus	Left	0.763
	Right	0.739
Uncinate Fasciculus	Left	0.544
	Right	0.581
FIT MEASURE		
CFI		0.958
RMSEA		0.071

NOTE. Factor loadings are for mean FA in each white matter tract. CFI = Comparative Fit Index, RMSEA = Root Mean Squared Error of Approximation.

Image Quality Assurance

Raw image quality was assessed with both a visual inspection and with automated software. For the visual inspection, maps of the sum of squares error (SSE) of the tensor fit were inspected for structured signal that is consistent with motion and other artifacts in the diffusion-weighted images (e.g., attenuated slices in diffusion-weighted images), and datasets determined to be of poor quality were excluded (n=109, ~10%). In addition to this visual inspection, slice-wise signal intensity was examined for attenuation resulting from motion, cardiac pulsation and other artifacts using the automated DTIprep quality control tool (http://www.nitrc.org/projects/dtiprep/, see Supplementary Material for further details). An additional 146 (14%) datasets were excluded based on the DTIprep results, leaving 778 DTI datasets for analysis.

Probabilistic tractography data were inspected visually in two ways. First, the native space FA map to FMRIB-58 FA space registration was inspected, to ensure images were all properly aligned to the

template (and thus seed / target / etc. masks were properly mapped to native space). Second, all tracts were visualized to ensure accurate path reconstruction.

Missing Data

Neuropsychological assessments were missing in 11 children (1.5%). Further, in some datasets, fiber tracts were not reconstructed. Specifically, data were missing in the left (n = 31, 4%) and the right (n = 15, 2%) cingulum bundle. This is likely the result of a relatively small fiber bundle coupled with the comparatively large spatial resolution of the DTI data. Lastly, from the age-6 assessment wave, data on behavioral problems were missing in 58 children (8%), and IQ was not available in 59 children (8%). A description of how missing data were handled is provided below.

Statistical Analysis

Statistical analyses were performed using the R Statistical Software version 3.1.0 (R Core Team, 2014). Structural equation modeling (SEM) was used to model associations between DTI scalar measures and cognitive domain scores (lavaan, Rosseel, 2012). Cognitive variables were entered into the model as the dependent variable and latent variables constructed from DTI measures were entered as the independent variable (described below). In order to mitigate confounding effects, models were also adjusted for covariates, namely age, sex, and the total behavioral problems score measured by the CBCL. In a second step for models including the NEPSY domain scores, non-verbal IQ was added to determine the specificity of the structure-function associations. In order to ensure that children with behavioral problems were not responsible for driving associations, additional sensitivity analyses were run in a subgroup of children who scored below the clinical cutoff on all CBCL scales (Tick et al., 2007). Lastly, the 'group' function in lavaan was used to determine whether boys and girls showed different structure-function associations. To test the presence of such an interaction effect of sex and FA on cognition, the multi-group feature of lavaan was used with sex entered as the grouping factor. The model was first run allowing the FA regression coefficient to vary between boys and girls, and a second time where the FA regression coefficient was estimated for boys and girls together. The $\chi 2$ difference between these models was computed, and the p-value for the difference was obtained from the standard $\chi 2$ distribution.

An illustration of the general modeling strategy is depicted in Figure 6.1. Goodness of fit was judged based on the Comparative Fit Index (CFI) and the Root Mean Square Error of Approximation (RMSEA). While it has also been shown that strict cutoff values of these fit indices are generally not recommended, a guide of a CFI > 0.95 and an RMSEA < 0.06 indicate good model fit (Hu and Bentler, 1999). To address the issue of missing data, the full-information maximum likelihood estimator provided by the lavaan package was implemented. In order to further limit the number of statistical tests, a hierarchical approach was utilized where only FA was examined in the initial SEM models. In cases where there was a significant association between FA and neuropsychological performance, follow-up SEM models were run using MD, AD, and RD.

Latent DTI Predictors and Confirmatory Factor Analyses

Within the SEM framework, latent variables were modeled from the DTI data and used in regression models to predict cognitive performance. While limited evidence to date suggests that associations

between white matter microstructure and cognitive performance are limited to focal brain regions or to a particular set of tracts, it is also possible that intelligence and certain aspects of cognition are global, and related to many tracts. Within this construct, a hierarchical approach was used to examine associations between white matter microstructure and cognition. First, to assess whether global measures of white matter integrity predict cognitive performance, a number of tracts commonly reported in the literature were combined into a single latent factor ("global factor"). In order to ensure that the global DTI latent predictor was statistically valid, a confirmatory factor analysis (CFA) was run. As can be seen in Table 6.1, model fit was within acceptable limits, suggesting the global factor can be used to model the association between white matter and cognition. For the second part of this hierarchical approach, to hone in on specificity of tracts, multiple linear regressions were run on the individual tracts when the global analysis yielded a significant association between FA and cognitive performance. The multiple linear regressions were set up identical to the regressions used in SEM analyses, except individual tract metrics were used as opposed to latent DTI variables. Given the number of regressions run in these post-hoc tests, correction for multiple comparisons was employed by first computing the effective number of statistical tests (Galwey, 2009), and then employing a Šidák correction based on the effective number of independent tests, which yields a new alpha value required for statistical significance (Šidák, 1967).

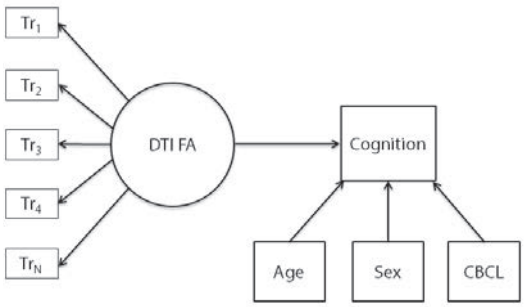

FIGURE 6.1. Outline of general structural equation modeling strategy.
Tr denotes the various tracts comprising the latent factor, and CBCL = Child Behavior Checklist total sum score.

RESULTS

Demographics

Children were on average 7.99 ± 1.02 years old, and boys (n = 401) and girls (n = 377) did not differ in age (t(774) = 0.98, p=0.33). Average non-verbal IQ in the sample was 102.5 ± 14.3. Additional demographic and descriptive details are presented in Table 6.2, including information on the smaller, sensitivity analysis sample (n = 521).

TABLE 6.2. **SAMPLE DESCRIPTIVE INFORMATION**

	FULL SAMPLE n = 778	SENSITIVITY ANALYSES n = 521
Age of MRI	7.99 ± 1.02	7.96 ± 1.02
Sex (F / M)(%)	377 (48) / 401 (52)	255 (49) / 266 (51)
IQ	102.5 ± 14.3	103.6 ± 14.03
Ethnicity (%)		
• Dutch	543 (70)	349 (76)
• Western	56 (7)	37 (7)
• Non-Western	179 (23)	90 (17)

NOTE. Sensitivity analyses include subjects who score below the clinical cutoff for behavioral problems. IQ = non-verbal intelligence quotient.

IQ

Results from SEM analyses using a global FA factor are presented in Table 6.3. A significant positive association was observed between IQ and FA (Standardized Estimate = 0.12, p = 0.005). Latent DTI measures of diffusivity were also created and IQ was significantly positively associated with AD (Standardized Estimate = 0.10, p = 0.014) (Supplementary Table S6.1). In order to further determine if all or only some of the tracts contribute to these effects, a hierarchical approach using univariate regression analyses was used (Supplementary Table S6.2). After adjusting for multiple comparisons, FA in the left superior longitudinal fasciculus (SLF) (β = 0.13, p = 0.002) and bilateral uncinate fasciculus (UF) (Left: β = 0.12, p = 0.002, Right: β = 0.17, p < 0.001) showed a positive association with IQ. Further, AD in the bilateral UF also showed a significant positive association with IQ (Left: β = 0.13, p = 0.001, Right: β = 0.12, p = 0.002). While not statistically significant after adjusting for multiple comparisons, the FA in the right SLF was also associated with IQ (β = 0.12, p = 0.003).

Neuropsychological Performance

Table 6.3 shows the analyses examining DTI associations with neuropsychological performance. Only the visuospatial domain was significantly associated with FA (Standardized Estimate = 0.13, p = 0.001). Follow-up analyses of diffusivity (Supplementary Table S6.1) showed RD was significantly associated with visuospatial ability (Standardized Estimate = -0.08, p = 0.03). In order to further investigate associations, tracts comprising the global DTI latent predictor were individually submitted to linear regression analysis, similar to what was done with IQ (Supplementary Table S6.3). After adjusting for multiple comparisons, FA in the left inferior longitudinal fasciculus (ILF) (β = 0.12, p = 0.001), right SLF (β = 0.12, p = 0.001), and right UF (β = 0.11, p = 0.002) showed a significant positive association with visuospatial ability, and RD in the right SLF showed a significant negative association (β = -0.12, p = 0.001). While not statistically significant after adjusting for multiple comparisons, many of the tracts in the contralateral hemisphere corresponding to those reported above showed marginally significant associations with visuospatial ability (see Supplementary Table S6.3).

TABLE 6.3. **ASSOCIATIONS BETWEEN FRACTIONAL ANISOTROPY AND COGNITION**

	REGRESSION SUMMARY					FIT MEASURES	
OUTCOME	B	SE	95% CI	STANDARDIZED EST.	P	CFI	RMSEA
Non-Verbal IQ	0.588	0.209	0.178, 0.998	0.120	**0.005**	0.910	0.077
Total NEPSY	0.011	0.010	-0.009, 0.031	0.038	0.284	0.917	0.075
Att. & EF	0.002	0.011	-0.020, 0.024	0.006	0.879	0.914	0.075
Language	-0.002	0.012	-0.026, 0.021	-0.007	0.850	0.915	0.076
Mem. & Learn.	0.011	0.011	-0.011, 0.033	0.037	0.322	0.915	0.076
Sens. Motor	0.007	0.013	-0.019, 0.033	0.021	0.614	0.911	0.076
Vis. Spatial	0.040	0.012	0.016, 0.063	0.129	**0.001**	0.915	0.076

NOTE. Models adjusted for age, sex and behavioral problems. CFI = Comparative fit index, 95% CI = confidence interval of B, SE=standard error of B, Standardized Est. = Standardized regression estimate, RMSEA =Root Mean Squared Error of Approximation. Att. & EF = Attention & Executive Function, Mem. & Learn = Memory & Learning, Sens. = Sensory, Vis. = Visual. Significant results are **bold**.

Sensitivity Analyses

In order to confirm that data from children with higher levels of problem behavior were not driving the observed associations, SEM sensitivity analyses were run excluding these children who scored in the clinical range on any CBCL scale. Results in these 521 children remained consistent for both IQ (standardized estimate = 0.12, p = 0.02) and for the visuospatial domain (standardized estimate = 0.13, p = 0.004).

Effects of Age, Sex and IQ

For illustrative purposes, the effect of age in the structure-function associations is presented. First, the association between age and the global FA latent factor is highly significant (Standardized Estimate = 0.32, p < 1 x 10-15). Second, to further visualize the effect of age, Supplementary Table S6.4 shows the structure-function associations when age is not entered in the models, and all neuropsychological domains except for the sensorimotor domain show a significant association with FA.

As previous research has shown differential developmental trajectories in white matter for boys and girls, it was of interest to determine whether the structure-function associations varied similarly. Results from testing for FA-by-sex interactions are presented in Table 6.4. For all cognitive domains the interaction effect was non-significant, indicating the association is equivalent in boys and girls.

Lastly, in order to rule out that the associations with visuospatial ability were the result of general intellectual ability, child IQ was also entered into the SEM models. While the effect estimates are lower, the structure-function association remained significant (Standardized Estimate = 0.09, p = 0.01), suggesting some specificity in the association between white matter integrity and visuospatial ability.

TABLE 6.4. **TEST OF INTERACTION EFFECT OF SEX AND FA ON COGNITION**

	χ^2	P
Non-Verbal IQ	1.983	0.159
Total NEPSY	0.881	0.348
Att. & EF	0.333	0.564
Language	1.756	0.185
Mem. & Learn.	0.020	0.886
Sens. Motor	0.944	0.331
Vis. Spatial	2.534	0.111

NOTE. Table shows χ^2 difference in models where multi-group SEM is used with and without constraining the regression coefficient for FA for boys and girls.

DISCUSSION

The current study demonstrates the presence of associations between cognition and white matter integrity in a large sample of young children. Specifically, we observed associations between general intellectual functioning, assessed through non-verbal IQ, and white matter integrity. In addition, visuospatial ability was associated with white matter integrity independent of age, sex, and general intellectual functioning. Both IQ and visuospatial ability were positively associated with FA. In terms of general direction of association, these findings are largely consistent with previous work in developmental age-association studies examining white matter microstructure. Interestingly, while the data show evidence for somewhat global effects across multiple white matter fiber bundles, more focused analyses suggest the associations are potentially driven by a sub-set of white matter tracts in the brain. Lastly, despite limited evidence of differential developmental effects for boys and girls in the literature, no evidence for differential structure-function associations was found in the present study.

Previous work in children has demonstrated associations between white matter microstructure and IQ (Navas-Sanchez et al., 2014; Schmithorst et al., 2005). Schmithorst et al. (2005) used a voxel-based approach to show positive associations between IQ and FA in multiple regions, particularly association bundles connecting frontal, parietal and occipital lobes. The same study also showed negative associations between IQ and MD, mostly in frontal white matter. Using voxel-based and region-of-interest methods, Navas-Sanchez et al. (2014) found associations between IQ and FA, primarily in the corpus callosum. In the present study, the association fibers included in these analyses, especially the SLF, overlap well with voxel-wise clusters observed in Schmithorst et al. (2005). Of importance, the models were adjusted for age (among other covariates), demonstrating the structure-function relationship is independent of the age-related developmental effects previously reported (Schmithorst and Yuan, 2010).

From the neuropsychological test battery administered in the current study, only the visuospatial domain was significantly associated with white matter integrity. Similar to what was observed for IQ, these associations were independent of age. Of importance, a considerable amount of variability in white matter microstructure is explained by age, even in this restricted age-range; thus, such a structure-function association that is independent of age is of interest. Further, when the child's non-verbal

IQ was added to the model, the association remained significant, suggesting specificity in the association between white matter microstructure and visuospatial ability. This is interesting given the measure of non-verbal IQ taps a similar construct as the visuospatial domain. Few studies have examined associations between white matter and visuospatial ability in typically developing children. Fryer et al. (2008) examined the corpus callosum in adolescents and found a relationship between white matter microstructure and visuospatial ability. Not surprisingly, a fair bit of work in this area has been conducted in children with autism (McGrath et al., 2013; Sahyoun et al., 2010). For instance, white matter microstructure in the SLF has been positively related to visuospatial ability in controls, but not in high-functioning children with autism (Sahyoun et al., 2010). In another study of young adults with autism, disruptions in the inferior fronto-occipital fasciculus were related to problems in visuospatial processing ability (McGrath et al., 2013).

A hierarchical approach was taken to examine whether certain tracts contributed more to the associations outlined above, and two tracts were found to be associated with non-verbal IQ after adjusting for multiple comparisons: the SLF and the UF. For the visuospatial domain, the ILF, SLF and UF were all found to be associated with visuospatial ability. Interconnecting the temporal and occipital lobes, the ILF is an association bundle that is believed to be involved in many tasks, such as visual object recognition and memory (Schmahmann et al., 2007). The SLF, primarily linking the frontal and parietal lobes, has been implicated in numerous functions, including motor, spatial, attention and language activities (Schmahmann et al., 2007). The UF, which primarily links rostral temporal areas to the orbitofrontal region, has been shown to be involved in aspects of both cognition and emotion, including learning, memory, recognition, and behavioral regulation (Schmahmann et al., 2007). Taken together, it is not surprising that a metric of general intellectual function is related to multiple fiber paths subserving a variety of cortical gray regions responsible for a myriad of cognitive processes. It is also reasonable that the more specific cognitive domain of visuospatial ability is associated with a specific tract. This tract, the SLF, has also been shown to be associated with visuospatial ability in children with and without psychiatric problems (Sahyoun et al., 2010).

While the visuospatial cognitive domain was associated with white matter, the other domains (Attention and Executive Function, Language, Learning and Memory, and Sensorimotor) were not. Previous work has shown associations between white matter integrity and these other cognitive abilities (Ge et al., 2013; Klarborg et al., 2013; Mabbott et al., 2009; Muetzel et al., 2008; Qiu et al., 2008), though considerable factors that could lead to this inconsistency are plausible. One possible explanation is that individual differences in brain development interfere with our ability to detect certain structure-function associations. As an example, consider that the heritability of white matter volume increases with age (Wallace et al., 2006), and that this is the result of a complex interplay between various genetic and environmental factors. The presence of age-related heritability may indicate some individual differences in the process of white matter maturation, even within a narrow age-range, leading to differences in timing and rate of development. Such a process can potentially explain the absence of structure-function associations in children, which then perhaps stabilize later in life. Along similar lines, other studies examining brain-behavior associations typically do so with a larger age-range, suggesting residual age-effects could be contributing to the observed structure-function associations. In the current study, all domains except for the sensorimotor show significant associations with white matter when models are not adjusted for age (Supplementary Table S6.4).

The current study does not show evidence for differential associations between white matter integrity and cognition in boys and girls. Previous work has shown sex differences, both in brain development and in behavioral and cognitive associations (Hanggi et al., 2010; Schmithorst, 2009; Schmithorst et al., 2008; Simmonds et al., 2014; Wang et al., 2012). One potential explanation for the lack of an interaction effect in the present study is that the effects are specific rather than global. In all analyses, the present study examined global domains of cognition, as well as global latent factors of white matter integrity. It is possible there are differential structure-function effects in boys and girls, however we do not observe them because the measures are too broad.

Despite the extensive application of DTI in the study of white matter status for more than a decade, limited information is available on precisely how the underlying neurobiology contributes to the MR-diffusion profile. However, some early studies do offer insight into this important question. For instance, it has been demonstrated that myelin is not a requisite of anisotropic diffusion (Beaulieu and Allen, 1994). While previous work has shown that axonal structure and packing are likely the main determinates of the diffusion profile observed by DTI, myelin does play a role in modulating diffusion anisotropy (Beaulieu, 2002; Gulani et al., 2001). Interestingly, perpendicular diffusion and trace diffusion are higher in the absence of myelin. Thus, in the context of the current study, one may postulate that associations between IQ and AD are potentially the result of axonal packing and structure, whereas the associations between visuospatial ability and RD are perhaps related to myelination. This is only one of many possibilities, given the complexities associated with disentangling the various cellular contributions to the diffusion signal.

One obvious strength of the current study is the large sample size. This allows us to adjust for multiple important variables (i.e., behavioral problems, child IQ, and maternal IQ) and to also conduct analyses in boys and girls separately, while maintaining a relatively high level of power. In addition to the large sample size, we focused our recruitment effort to children within a relatively restricted age range. This reduces potential confounding, and increases our ability to focus more on associations with cognition and less on age-related developmental effects. Further, the current study uses a probabilistic tractography approach, providing native-space information on white matter tracts, which is not sensitive to common problems associated with voxel-based analyses (e.g., misalignment). Another strength of the study is the use of SEM. In particular, one appealing feature of SEM is the ability to estimate latent variables from multiple predictors, which can then be used in standard regression models. This is an elegant approach to data reduction that should be explored further in tract-based analyses, where numerous tracts and scalar metrics are available for analysis. Not only does this limit the number of statistical tests and Type-I error, but also gives future work a guide for more focal hypotheses.

The current study is not without limitations. Importantly, the associations presented here are cross-sectional, and thus we cannot rule out reverse causality. While the Generation R Study is now conducting MRI scanning on all children during the 10-year assessment and will eventually have longitudinal data, we currently only have a single time-point. Another potential limitation is the separation in time between the assessment of non-verbal IQ and the MRI scan (on average 1.8 years apart). Even though non-verbal intelligence and white matter microstructure were assessed at separate visits nearly two years apart, a robust association between white matter and IQ was observed. Thus, the associations for IQ and white matter observed in this study are likely underestimates of the true association. Further, as the sample used in the present study is a sub-sample of the larger population-based study

that oversampled children with specific behavioral problems, it is reasonable to question whether our estimates of cognition are representative in the general population. However, as can be seen in Table 6.2, average IQ in the present study was consistent with the normalized distribution (mean of 100, standard deviation of 15). Lastly, there is still potential for residual confounding in the present study. One possible source could be age-related. While we attempted to mitigate these effects with a narrow age-range and by using age as a covariate, we cannot rule out the potential for residual confounding of age. For the associations with IQ, previous work has shown relative stability of IQ estimates over time, suggesting a structure-function association between FA and IQ would not be confounded by age.

To conclude, the current study demonstrates that non-verbal intelligence and visuospatial ability are associated with white matter microstructure in children ages 6-to-10 years old. Such structure-function associations are useful in improving our understanding of brain maturation and cognitive development, and may even one day become a viable clinical utility to aid in diagnosis, prognosis and treatment of neurological and psychiatric disorders. The current study focuses on broad domains of cognitive function, and future work should explore the specific components that make up non-verbal IQ and visuospatial ability. Further, it will be of interest to explore potential cognitive associations in resting-state functional MRI, and perhaps even multi-modal metrics utilizing both functional and structural connectivity (Sui et al., 2012).

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SUPPLEMENT

Image Quality Assurance

Raw image quality was assessed with both a visual inspection and with automated software. For the visual inspection, the sum of squares error (SSE) of the tensor fit for each voxel was written out in Nifti image format. The SSE maps were inspected for structured signal that is consistent with motion and other artifacts in the diffusion-weighted images (e.g., attenuated slices in diffusion-weighted images), and were rated from 0-to-3, with 0 = none, 1 = minimal issues, 2 = moderate issues and 3 = severe issues. Cases rated as having severe issues in the SSE map were immediately excluded. In addition to this visual inspection, slice-wise signal intensity was examined for attenuation resulting from motion, cardiac pulsation and other artifacts using the automated DTIprep quality control tool (http://www.nitrc.org/projects/dtiprep/). From the DTIprep output, an overall quality metric was computed from the total affected slices and volumes as follows:

Equation 1:

$$Q = \frac{V}{35} \cdot \left(S_t + \sum_{i \in V_O} (S_{\cap i} \cdot i) \right)$$

where Q is the overall quality score, V is the total number of affected volumes, 35 being the total number of diffusion weighted volumes, S_t is the total number of affected slices, V_O is the number of volumes where affected slices are overlapping (0, 2, 3, or 4), and $S\cap$ is the number of overlapping affected slices across VO volumes. Thus, a high overall quality score corresponds to data with more artifacts / motion. Further, the second portion of the equation includes a term to account for datasets with the same slice(s) affected across multiple volumes. In total, 109 (10%) datasets were excluded as a result of being flagged as having a SSE map with severe problems. In order to estimate a reasonable exclusionary cut-off for the automated overall quality measure derived from DTIprep, the quality measure was associated with a whole-brain FA value. The whole-brain FA value was computed for each dataset by averaging all voxels within the FMRIB-58 FA "skeleton", which is a commonly used white matter mask (Smith et al., 2006). At various thresholds of the overall quality measure, Pearson correlation coefficients were computed for the association between the overall quality measure and mean skeleton FA. A cutoff value for the automated overall quality measure was determined as the point at which there is not a significant association between FA and data quality. At this threshold, an additional 146 (14%) datasets were excluded, leaving 778 DTI datasets for analysis.

Probabilistic tractography data were inspected visually in two ways. First, the native space FA map to FMRIB-58 FA space registration was inspected, to ensure images were all properly aligned to the template (and thus seed / target / etc. masks were properly mapped to native space). Second, all tracts were visualized to ensure accurate path reconstruction.

TABLE S6.1. **ASSOCIATIONS BETWEEN DIFFUSIVITY MEASURES AND COGNITION**

		REGRESSION SUMMARY					FIT MEASURES	
OUTCOME	PREDICTOR	B	SE	95% CI	STANDARDIZED EST.	P	CFI	RMSEA
Non-verbal IQ	MD	0.061	0.260	-0.449, 0.571	0.010	0.814	0.935	0.068
	AD	0.537	0.218	0.110, 0.964	0.102	**0.014**	0.944	0.056
	RD	-0.216	0.198	-0.604, 0.171	-0.046	0.273	0.925	0.073
Vis. Spatial	MD	-0.014	0.015	-0.043, 0.016	-0.033	0.369	0.934	0.069
	AD	0.020	0.012	-0.004, 0.045	0.060	0.106	0.948	0.054
	RD	-0.025	0.011	-0.048 ,-0.003	-0.083	**0.028**	0.925	0.074

NOTE. Predictors, MD=Mean Diffusivity, AD=Axial Diffusivity, RD=Radial Diffusivity are latent factors using the same tracts outlined in Table 6.1. CFI = Comparative fit index, 95% CI = confidence interval of B, RMSEA =Root Mean Squared Error of Approximation, SE=standard error of B, Standardized Est. = Standardized regression estimate. Significant results are **bold**.

TABLE S6.2. **UNIVARIATE ASSOCIATIONS BETWEEN NON-VERBAL IQ AND WHITE MATTER**

TRACT	DTI	B	95% CI	β	P	R^2
L CB	FA	0.077	-0.175, 0.330	0.025	0.548	0.018
	MD	-0.247	-0.608, 0.115	-0.055	0.181	0.020
	AD	-0.069	-0.302, 0.163	-0.024	0.558	0.018
	RD	-0.154	-0.419, 0.111	-0.047	0.254	0.020
R CB	FA	-0.054	-0.312, 0.205	-0.017	0.683	0.016
	MD	0.004	-0.369, 0.377	0.001	0.982	0.016
	AD	-0.052	-0.283, 0.178	-0.018	0.655	0.016
	RD	0.042	-0.237, 0.321	0.012	0.767	0.016
L CST	FA	0.167	-0.237, 0.571	0.033	0.418	0.017
	MD	0.132	-0.309, 0.574	0.023	0.557	0.017
	AD	0.199	-0.109, 0.508	0.049	0.205	0.019
	RD	-0.003	-0.351, 0.345	-0.001	0.985	0.016
R CST	FA	0.460	0.053, 0.867	0.088	0.027	0.024
	MD	0.009	-0.416, 0.435	0.002	0.966	0.016
	AD	0.298	-0.012, 0.609	0.072	0.059	0.022
	RD	-0.170	-0.510, 0.171	-0.038	0.328	0.018
FMa	FA	0.311	-0.002, 0.624	0.076	0.052	0.022
	MD	-0.001	-0.189, 0.187	0.000	0.991	0.016
	AD	0.079	-0.073, 0.232	0.040	0.306	0.018
	RD	-0.054	-0.229, 0.121	-0.024	0.546	0.017
FMi	FA	0.219	-0.118, 0.556	0.049	0.202	0.019
	MD	0.148	-0.136, 0.431	0.039	0.307	0.018
	AD	0.168	0.009, 0.327	0.079	0.038	0.023
	RD	-0.039	-0.303, 0.226	-0.011	0.774	0.016
L ILF	FA	0.550	0.057, 1.042	0.086	0.029	0.023
	MD	-0.179	-0.564, 0.207	-0.037	0.364	0.017
	AD	0.072	-0.209, 0.354	0.020	0.614	0.017
	RD	-0.297	-0.659, 0.065	-0.066	0.108	0.020

TABLE S6.2. **UNIVARIATE ASSOCIATIONS BETWEEN NON-VERBAL IQ AND WHITE MATTER** (CONTINUED)

TRACT	DTI	B	95% CI	β	P	R^2
R ILF	FA	0.726	0.250, 1.203	0.120	0.003	0.030
	MD	-0.036	-0.421, 0.349	-0.007	0.854	0.016
	AD	0.260	-0.005, 0.525	0.074	0.054	0.022
	RD	-0.291	-0.654, 0.072	-0.063	0.116	0.020
L SLF	**FA**	**0.716**	**0.269, 1.163**	**0.128**	**0.002**	**0.031**
	MD	0.014	-0.417, 0.445	0.003	0.948	0.016
	AD	0.429	0.080, 0.778	0.093	0.016	0.025
	RD	-0.231	-0.606, 0.145	-0.049	0.228	0.018
R SLF	FA	0.703	0.244, 1.162	0.120	0.003	0.030
	MD	-0.006	-0.428, 0.415	-0.001	0.976	0.016
	AD	0.350	0.015, 0.686	0.079	0.040	0.023
	RD	-0.231	-0.610, 0.148	-0.047	0.232	0.018
L UF	**FA**	**0.573**	**0.211, 0.935**	**0.123**	**0.002**	**0.031**
	MD	0.060	-0.367, 0.486	0.011	0.783	0.016
	AD	**0.542**	**0.224, 0.860**	**0.128**	**0.001**	**0.033**
	RD	-0.231	-0.559, 0.097	-0.056	0.167	0.019
R UF	**FA**	**0.974**	**0.543, 1.404**	**0.171**	**0.000**	**0.045**
	MD	-0.169	-0.634, 0.297	-0.028	0.477	0.017
	AD	**0.489**	**0.176, 0.801**	**0.119**	**0.002**	**0.030**
	RD	-0.570	-0.963, -0.176	-0.111	0.005	0.028

NOTE. Analyses adjusted for age, sex, and behavioral problems. New alpha-level, accounting for multiple testing is 0.003. Significant results are **bold**. CB=cingulum bundle, CST=corticospinal tract, FMa=forceps major, FMi=forceps minor, ILF=inferior longitudinal fasciculus, SLF=superior longitudinal fasciculus, UF=uncinate fasciculus, R=right, L=left

TABLE S6.3. **UNIVARIATE ASSOCIATIONS BETWEEN VISUOSPATIAL ABILITY AND WHITE MATTER**

TRACT	DTI	B	95% CI	β	P	R^2
CB L	FA	0.008	-0.007, 0.022	0.039	0.285	0.157
	MD	-0.004	-0.025, 0.017	-0.015	0.680	0.156
	AD	0.006	-0.007, 0.019	0.033	0.357	0.157
	RD	-0.008	-0.023, 0.008	-0.037	0.324	0.157
CB R	FA	0.012	-0.003, 0.026	0.056	0.122	0.155
	MD	-0.010	-0.031, 0.012	-0.034	0.364	0.153
	AD	0.004	-0.009, 0.018	0.023	0.513	0.152
	RD	-0.011	-0.027, 0.004	-0.053	0.158	0.154
CST L	FA	0.019	-0.004, 0.043	0.059	0.111	0.157
	MD	-0.007	-0.033, 0.018	-0.020	0.576	0.154
	AD	0.009	-0.009, 0.026	0.033	0.339	0.155
	RD	-0.012	-0.033, 0.008	-0.044	0.227	0.156
CST R	FA	0.015	-0.009, 0.039	0.044	0.220	0.156
	MD	-0.007	-0.032, 0.017	-0.020	0.559	0.154

TABLE S6.3. **UNIVARIATE ASSOCIATIONS BETWEEN VISUOSPATIAL ABILITY AND WHITE MATTER** (CONTINUED)

TRACT	DTI	B	95% CI	β	P	R^2
	AD	0.005	-0.013, 0.024	0.021	0.553	0.154
	RD	-0.010	-0.030, 0.009	-0.036	0.305	0.155
FMa	FA	0.024	0.006, 0.043	0.093	0.009	0.162
	MD	-0.004	-0.014, 0.007	-0.024	0.498	0.154
	AD	0.005	-0.004, 0.013	0.036	0.298	0.155
	RD	-0.008	-0.018, 0.002	-0.055	0.119	0.157
FMi	FA	0.004	-0.015, 0.023	0.014	0.675	0.154
	MD	0.017	0.000, 0.033	0.068	0.047	0.159
	AD	0.011	0.002, 0.020	0.080	0.022	0.160
	RD	0.007	-0.009, 0.022	0.029	0.398	0.155
ILF L	FA	**0.049**	**0.021, 0.077**	**0.120**	**0.001**	**0.168**
	MD	-0.016	-0.039, 0.006	-0.053	0.162	0.156
	AD	0.008	-0.009, 0.024	0.034	0.348	0.155
	RD	-0.028	-0.049, -0.007	-0.097	0.010	0.162
ILF R	FA	0.037	0.009, 0.064	0.094	0.008	0.162
	MD	0.002	-0.020, 0.025	0.007	0.836	0.154
	AD	0.017	0.002, 0.032	0.075	0.030	0.160
	RD	-0.013	-0.034, 0.008	-0.044	0.233	0.156
SLF L	FA	0.034	0.008, 0.059	0.095	0.009	0.162
	MD	-0.008	-0.033, 0.017	-0.022	0.541	0.154
	AD	0.016	-0.004, 0.036	0.054	0.123	0.157
	RD	-0.018	-0.040, 0.004	-0.059	0.106	0.157
SLF R	FA	**0.046**	**0.020, 0.072**	**0.122**	**0.001**	**0.168**
	MD	-0.029	-0.054, -0.005	-0.082	0.020	0.160
	AD	0.004	-0.015, 0.024	0.015	0.664	0.154
	RD	**-0.037**	**-0.059, -0.016**	**-0.119**	**0.001**	**0.167**
UF L	FA	0.030	0.010, 0.051	0.102	0.004	0.164
	MD	-0.012	-0.036, 0.013	-0.034	0.357	0.155
	AD	0.019	0.000, 0.037	0.069	0.049	0.159
	RD	-0.020	-0.039, -0.001	-0.075	0.039	0.159
UF R	FA	**0.039**	**0.014, 0.065**	**0.108**	**0.002**	**0.165**
	MD	-0.010	-0.037, 0.017	-0.026	0.475	0.154
	AD	0.017	-0.002, 0.035	0.063	0.075	0.158
	RD	-0.024	-0.048, -0.001	-0.074	0.040	0.159

NOTE. Analyses adjusted for age, sex, and behavioral problems. New alpha-level, accounting for multiple testing is 0.003. Significant results are **bold**. CB=cingulum bundle, CST=corticospinal tract, FMa=forceps major, FMi=forceps minor, ILF=inferior longitudinal fasciculus, SLF=superior longitudinal fasciculus, UF=uncinate fasciculus, R=right, L=left

TABLE S6.4. **ASSOCIATIONS BETWEEN FRACTIONAL ANISOTROPY AND COGNITION, UNADJUSTED FOR AGE**

OUTCOME	B	SE	95% CI	STANDARDIZED EST.	P	CFI	RMSEA
Non-Verbal IQ	0.559	0.198	0.170, 0.947	0.114	**0.005**	0.943	0.064
Total NEPSY	0.061	0.012	0.038, 0.083	0.206	**$<1x10^{-6}$**	0.941	0.065
Att. & EF	0.034	0.011	0.012, 0.056	0.116	**0.003**	0.945	0.063
Language	0.049	0.013	0.024, 0.075	0.151	**$<1x10^{-3}$**	0.940	0.066
Mem. & Learn.	0.055	0.012	0.031, 0.079	0.178	**$<1x10^{-5}$**	0.943	0.064
Sens. Motor	0.008	0.013	-0.017, 0.033	0.025	0.526	0.944	0.064
Vis. Spatial	0.070	0.012	0.046, 0.094	0.225	**$<1x10^{-8}$**	0.944	0.064

NOTE. Models adjusted for sex and behavioral problems. CFI = Comparative fit index, 95% CI = confidence interval of B, SE=standard error of B, Standardized Est. = Standardized regression estimate, RMSEA =Root Mean Squared Error of Approximation. Att. & EF = Attention & Executive Function, Mem. & Learn = Memory & Learning, Sens. = Sensory, Vis. = Visual. Significant results are **bold**.

CHAPTER

7

Cortical morphology as a shared neurobiological substrate of attention-deficit/hyperactivity problems and executive functioning

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Submitted for publication

ABSTRACT

Background

Attention-deficit/hyperactivity problems have often been associated with poor cognitive functioning. Genetic studies have demonstrated a shared etiology of attention-deficit/hyperactivity disorder (ADHD) and cognitive ability, suggesting a common underlying neurobiology of ADHD and cognition. Further, neuroimaging studies suggest altered cortical development related to ADHD. The aim of our study was to investigate the role of cortical morphology, as potential shared underlying neurobiological substrate, in the association between attention-deficit/hyperactivity problems and cognitive problems.

Methods

In a population-based sample of 776 school-aged children data on attention-deficit/hyperactivity problems, cognitive functioning, and structural imaging data were collected. We investigated the association between attention-deficit/hyperactivity problems and different domains of cognition. The modulating role of cortical thickness and gyrification in any observed behavior-cognition association was studied.

Results

We found that attention-deficit/hyperactivity problems were related specifically to problems in attention and executive functioning (EF) (B=-0.041, p=0.004). Cortical thickness and gyrification were related to both attention-deficit/hyperactivity symptoms and EF in brain regions that have been previously implicated in ADHD, and partly explained the association between attention-deficit/hyperactivity problems and EF ($B_{indirect}$ = -0.008, BC 95% CI -0.0172;-0.0001).

Conclusions

In a large population-based sample of children, we identified a shared cortical morphology underlying attention-deficit/hyperactivity problems and EF.

INTRODUCTION

Neurodevelopmental disorders have often been associated with poor cognitive functioning and low levels of general intelligence in both clinical and epidemiological population-based samples (Basten et al., 2014; Dietz, Lavigne, Arend, & Rosenbaum, 1997). Studies of attention-deficit/hyperactivity disorder (ADHD) have shown moderate correlations between ADHD symptom scores and IQ scores and a significantly lower mean IQ in children with ADHD (Frazier, Demaree, & Youngstrom, 2004; Kuntsi et al., 2004). Yet, it is unclear whether these deficits in cognitive functioning represent a general cognitive deficit, or primarily reflect deficits in more specific cognitive domains. In order to parse out the specific cognitive problems, multiple studies have tested neuropsychological performance in clinical ADHD samples. These studies suggest that a wide range of neuropsychological domains is affected in patients with ADHD (Brodsky, Willcutt, Davalos, & Ross, 2014; Frazier et al., 2004; Willcutt, Doyle, Nigg, Faraone, & Pennington, 2005; Willcutt, Sonuga-Barke, Nigg, & Sergeant, 2008). Yet, two large meta-analyses (Frazier et al., 2004; Willcutt et al., 2005) demonstrated that ADHD seems most strongly related to tasks assessing executive functioning (EF).

Genetic studies have demonstrated a shared etiology of cognitive ability and child psychopathology in general and, more specifically, in ADHD (Kuntsi et al., 2004; Polderman et al., 2009). This shared genetic background suggests that a common neurobiology underlies ADHD and cognition. Previous neuroimaging studies in ADHD have shown a delay in brain maturation (Shaw et al., 2007) and a thinner cortex (Narr et al., 2009) throughout most of the cerebrum. In population-based pediatric samples, the latter association has also been demonstrated (Ducharme et al., 2012; Mous, Muetzel, et al., 2014). Studies of gyrification offer mixed results in children with ADHD. Some studies report a global decrease (Wolosin, Richardson, Hennessey, Denckla, & Mostofsky, 2009), while others report either a small, local increase (Mous, Karatekin, et al., 2014) or no abnormalities (Shaw et al., 2012). Based on the shared genetic background of ADHD and cognition, one might expect that the shared neurobiology underlying ADHD and cognition could potentially be reflected in cortical morphology. However, to our knowledge, no studies have assessed the role of cortical morphology in the association between ADHD symptoms and cognitive functioning.

The notion that child psychopathology, such as ADHD, is better described within a dimensional framework has recently gained support. Within this framework of continuous symptom levels, children with clinical disorders constitute the extreme end of the spectrum. Numerous studies demonstrate that such a dimensional approach can further contribute to a better etiological understanding of child psychopathology (Hudziak, Achenbach, Althoff, & Pine, 2007; Lubke, Hudziak, Derks, van Bijsterveldt, & Boomsma, 2009; Polderman et al., 2007; Shaw et al., 2011). For research purposes, the use of a continuous score provides more power and allows the application of more advanced statistical methods.

In the current large population-based study of school-aged children we aimed to investigate the role of cortical morphology in the association between ADHD symptoms and cognitive functioning. Based on previous studies, we selected two measures of cortical morphology that are shown to be implicated in ADHD; cortical thickness and gyrification. As these measures are regulated by different genetic mechanisms and tap different processes during development (Panizzon et al., 2009; Raznahan et al., 2011), this could provide additional knowledge regarding the underlying biology of attention-deficit/hyperactivity problems and cognition. We tested two hypotheses; first that ADHD symptoms would

be specifically associated with problems in EF, and second that cortical morphology (as the potential shared neurobiological substrate underlying both ADHD and cognitive problems) would explain this association.

MATERIALS AND METHODS

Participants

This study is embedded in the Generation R Study, a population-based cohort study in Rotterdam, the Netherlands (Jaddoe et al., 2012). When their child was around 6 years, the parents of 6,346 children reported on their child's behavior. In the same period, a brain Magnetic Resonance Imaging (MRI) study began within a subsample of the study. Between September 2009 and July 2013, a total of 1,325 children were recruited (White et al., 2013). As a part of this neuroimaging study, an extensive neuropsychological assessment was performed in 1,307 children. Of this group 1,053 children also had structural imaging data available and after quality control, 907 children remained. Data from the CBCL attention-deficit/hyperactivity problems scale were missing in 82 children of this group. Exclusion based on image quality was not related to the CBCL attention-deficit/hyperactivity problems score. Twins (n=17) and a randomly selected child from each sibling pair (n=11) were excluded. Finally, since attention-deficit/hyperactivity problems are known to be comorbid with autistic traits, all children with a score above the screening cut-off for autistic traits on the Social Responsiveness Scale (Constantino & Gruber, 2002) were excluded from the analyses (n=21). This resulted in a final study sample of 776 children. The study was approved by the Medical Ethics Committee (METC) of the Erasmus Medical Center. Written informed consent was obtained from the parents of all participants.

Attention-Deficit/Hyperactivity Problems

Attention-deficit/hyperactivity problems were measured with the DSM-oriented attention-deficit/hyperactivity problems scale score of the CBCL 1½-5 (Achenbach & Rescorla, 2000) at 6 years of age. All children were assessed with one instrument; the preschool CBCL was chosen because many children were younger than 6 years and older age versions are inappropriate for such young children. In the CBCL 1½-5, the primary caregiver is asked to answer 99 questions on a three-point scale regarding the behavior of their child, of which six items comprised the attention-deficit/hyperactivity problems scale. Good reliability and validity have been reported (Achenbach & Rescorla, 2000). Cronbach's alphas were similar in the 5-year-old children and in children of 6 years and older (α=0.80 and α=0.83 respectively), indicating that the attention-deficit/hyperactivity problems were reliably measured in the children older than 5 years of age.

Cognitive Functioning

Cognitive functioning was assessed as a part of the neuroimaging study, between 6 and 9 years of age, using a shortened version of the developmental NEuroPSYchological assessment (NEPSY-II-NL). The NEPSY-II-NL is a Dutch translation of the North-American NEPSY-II and assesses neuropsychological functioning in 5-to-12 year-old children, covering different domains of neuropsychological functioning, including Attention and Executive Functioning, Language, Memory and Learning, Sensorimotor Functioning and Visuospatial Processing (Korkman, Kirk, & Kemp, 2010).

In order to limit multiple testing, we analysed summary domainscores. Since the NEPSY-II-NL does not provide domainscores, we used the first unrotated factorscore of principal components analyses that we performed on the test scores comprising each predefined NEPSY-II-NL domain in all children in the brain imaging study with NEPSY-II-NL data (n=1,307) (Supplementary Table S7.1).

Cortical Morphology

MR images were acquired using a GE Discovery MR750 3.0 Tesla scanner (GE Healthcare Worldwide, Milwaukee, WI, USA) using an 8-channel head coil. A high-resolution T1-weighted image was collected using an IR-FSPGR sequence with the following parameters: TR=10.3ms, TE=4.2ms, TI=350ms, NEX=1, flip angle=16°, matrix 256x256, imaging acceleration factor of 2, and an isotropic resolution of 0.9x0.9x0.9mm^3. Cortical reconstruction was performed using the FreeSurfer image analysis suite (http://surfer.nmr.mgh.harvard.edu/), version 5.1 (Dale, Fischl, & Sereno, 1999). Image quality assurance was performed prior and after image processing (El Marroun, Schmidt, et al., 2014). Neuroimaging measures of interest were cortical thickness and gyrification.

Covariates

In Table 7.1, participant characteristics are presented. Child ethnicity was defined as Dutch if both parents were born in the Netherlands and as non-Dutch if at least one parent was born elsewhere (Statistics Netherlands, 2004). Maternal education was defined as highest education completed (Statistics Netherlands, 2004) and household income as the total net monthly income. Information on maternal alcohol use and smoking during pregnancy was obtained using questionnaires. Information on date of birth, gender, and birth weight was obtained from midwives and hospital registries. Gestational age was established using ultrasound measures during pregnancy. Non-verbal IQ of the child was assessed around 6 years of age, using a shortened version of the Snijders-Oomen Niet-verbale Intelligentie Test–Revisie (SON-R 2.5–7) (Tellegen, Winkel, Wijnberg-Williams, & Laros, 2005). The use of psychostimulant medication was recorded during the MRI visit. All covariates were selected based on their relevance according to the literature.

TABLE 7.1. **PARTICIPANT CHARACTERISTICS**

	TOTAL VALID OBSERVATIONS (n=776)	MEAN (SD)
CHILD CHARACTERISTICS		
Gender (% boys)	776	52.4
Ethnicity (%)	776	
• Dutch		70.8
• Other Western		6.8
• Non-Western		22.4
Age NEPSY-II-NL assessment, years	776	7.98 (0.98)
Age brain imaging, years	776	7.97 (0.99)
Age CBCL assessment, years	776	6.03 (0.40)
CBCL Attention-Deficit/Hyperactivity Problems, score	776	3.77 (2.90)
Non-verbal IQ, score	710	102.81 (14.34)
Gestational age at birth, weeks	775	39.97 (1.77)
Birth weight, grams	776	3470.41 (546.56)
Psychostimulant use (% yes)	753	2.9
MATERNAL CHARACTERISTICS		
Education level (%)	764	
• High		57.2
• Medium		31.0
• Low		11.8
Alcohol use during pregnancy (%)	722	
• Never		34.6
• Until pregnancy was known		14.3
• Continued occasionally during pregnancy		40.4
• Continued frequently during pregnancy[a]		10.7
Smoking during pregnancy (%)	749	
• Never		78.6
• Until pregnancy was known		6.3
• Continued during pregnancy		15.1
Household income (%)	743	
• >2000 euro		77.1
• 1200-2000 euro		17.0
• <1200 euro		5.9

NOTE. CBCL = Child Behavior Checklist; NEPSY = neuropsychological assessment. Values given as mean and standard deviation, unless otherwise indicated. [a] Frequent continued use was defined as one drink or more per week during at least 2 trimesters of pregnancy.

Statistical Analyses

Missing values of potential confounding factors were imputed using the multiple imputation (MCMC) method, with 5 imputations and 10 iterations. For 13 children, the local gyrification indices of the identified clusters could not be computed, therefore these were imputed using the folding index, mean curvature, Gaussian curvature, intrinsic curvature and global hemispheric gyrification index measures as estimates, which are equivalent or highly correlated to the local gyrification index. The CBCL attention-deficit/hyperactivity problems score and all NEPSY-II-NL scores were complete in all selected children. The CBCL attention-deficit/hyperactivity problems score, and the NEPSY-II-NL total score, Attention and Executive Functioning domainscore, and the Visuospatial Processing domainscore were square root transformed to approach a normal distribution. In all SPSS analyses, outcome and determinant were residualized for age, and covariates were added to the linear regression analyses.
The analyses were performed in three steps, as described below;

Cognitive functioning

First, to test our hypothesis that associations of ADHD symptoms with cognitive functioning would be specific to problems in EF, we performed linear regression analyses testing the association between the CBCL attention-deficit/hyperactivity problems score and the NEPSY-II-NL total- and domainscores.

Cortical morphology

In order to be able to test our second hypothesis that cortical morphology would explain the association between attention-deficit/hyperactivity problems and cognitive functioning, we studied the direct association between CBCL attention-deficit/hyperactivity problems and cortical morphology, as well as the direct association between NEPSY-II-NL cognitive functioning and cortical morphology (only for cognitive domain(s) that were associated with attention-deficit/hyperactivity problems). We performed whole-brain vertex-wise General Linear Model analyses using the FreeSurfer built-in module QDEC. Age during scanning and gender were included as covariates. To correct for multiple testing of all brain vertices, Monte Carlo Null-Z Simulations were performed using a threshold of 1.3 ($p<0.05$), controlling the rate of false positive clusters. Following the vertex-wise analyses in QDEC, we extracted the cortical thickness/gyrification data of significant cluster(s) for each individual and imported these into SPSS to test whether the association was potentially confounded by other factors by performing cluster-wise regression analyses with additional covariates. This analysis was performed only to assess confounding and should not be considered 'double-dipping'.

Interrelation cognitive functioning, cortical morphology and ADHD problems

Next, we tested our second hypothesis that cortical morphology is a shared neurobiological substrate underlying both ADHD and cognitive problems, and would thus explain at least part of the association between attention-deficit/hyperactivity problems and cognition. To this aim, we performed a multiple mediation analysis of the association between CBCL attention-deficit/hyperactivity problems with NEPSY-II-NL cognitive functioning by cortical morphology. The mediation analysis was performed using the 'indirect' macro in SPSS (http://www.afhayes.com/) with bias-corrected bootstrapping using 5,000 replications (Preacher & Hayes, 2008). A mediation analysis was chosen as statistical method because it allows us to assess cortical morphology as a potential shared biological substrate of at-

tention-deficit/hyperactivity problems and cognitive functioning. However, we made no assumptions about directionality in these associations.

We selected those cortical clusters as potential mediators that were both related to attention-deficit/hyperactivity problems and to cognitive functioning. To this aim, we performed retention/consistency analyses; clusters that were detected using the CBCL attention-deficit/hyperactivity problems score were tested for their relation with the NEPSY-II-NL score(s) and vice versa. Clusters were only retained and added as mediator in the respective mediation analysis if they also showed a significant or trend-level ($p<0.1$) association with the other measure.

RESULTS

Cognitive Functioning

After adjustment for all covariates, attention-deficit/hyperactivity problems were only associated with functioning in a single cognitive domain; Attention and Executive Functioning (β=-0.11, p=0.004). Children with more problems performed significantly worse on the tasks comprising this domain. No significant associations were found for any of the other cognitive domains (Supplementary Table S7.2).

Cortical Morphology

Detection of cortical clusters

Figure 7.1 and Table 7.2 (and Supplementary Table S7.3) show the association between attention-deficit/hyperactivity problems and cortical thickness. We detected five clusters in which more attention-deficit/hyperactivity problems were associated with a thinner cortex. The first cluster was located in the left caudalmiddlefrontal gyrus, encompassing parts of the rostralmiddlefrontal gyrus (β=-0.14, $p<0.001$). The second cluster was a large cluster in the right postcentral gyrus, spreading towards the precentral gyrus and the superiorparietal, superiortemporal and middletemporal gyri (β=-0.22, $p<0.001$). The third cluster was localized right lateraloccipital, spreading towards the inferiortemporal gyrus (β=-0.19, $p<0.001$). The fourth cluster consisted of the right superiortemporal gyrus (β=-0.16, $p<0.001$) and the fifth was localized right occipital (β=-0.15, $p<0.001$).

Vertex-wise cortical analysis did not show an association between cortical thickness and performance in the NEPSY-II-NL domain Attention and Executive Functioning after correction for multiple testing.

Figure 7.1 and Table 7.3 (see also Supplementary Table S7.3) show the association between attention-deficit/hyperactivity problems and gyrification. We detected three large clusters in which more attention-deficit/hyperactivity problems were related to less gyrification. Because the clusters were large and comprised different lobes and both lateral and medial regions of the brain, we provide a global label for each cluster. The first left hemisphere cluster (LH1) was a large cluster, covering parts of the frontal, temporal and parietal regions of the brain (β=-0.14, $p<0.001$). The second cluster (LH2) was localized left superiorparietal/postcentral (β=-0.11, p=0.006). The right hemisphere cluster (RH1) covered frontal, temporal and parietal areas of the brain (β=-0.13, p=0.001).

Figure 7.1 and Table 7.3 (see also Supplementary Table S7.3) show the association between NEPSY-II-NL Attention and Executive Functioning and gyrification. We detected five clusters in which worse functioning on the NEPSY-II-NL Attention and Executive Functioning domain was related to a lower local gyrification index. The first cluster (LH3) was located left inferiorparietal (β=0.08, p=0.03). The second cluster (LH4) covered a part of the left frontal area (β=0.09, p=0.02). The first cluster in the right hemisphere (RH2) was a large cluster covering the parietal lobe and extending into the frontal lobe (β=0.11, p=0.004). The second right hemisphere cluster (RH3) covered parts of frontal and temporal areas (β=0.07, p=0.07). Another cluster (RH4) was located in the right occipital lobe (β=0.08, p=0.02).

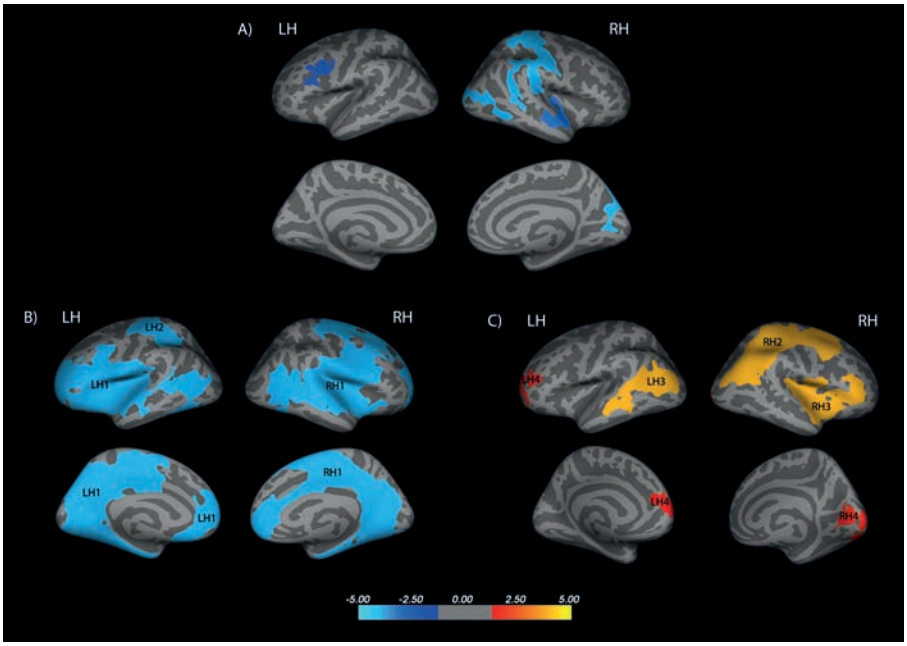

FIGURE 7.1. Significant clusters vertex-wise associations of A) CBCL attention-deficit/hyperactivity problems score and cortical thickness, B) CBCL attention-deficit/hyperactivity problems score and local gyrification index, and C) NEPSY-II-NL Attention and Executive Functioning score and local gyrification index. LH = left hemisphere, RH = right hemisphere. Colors represent the -log10(p-value). Blue clusters indicate a negative relation, showing a thinner cortex/less gyrification in relation to more problems. The yellow/red clusters indicate a positive relation, showing more gyrification in relation to better functioning.

Retention of cortical clusters

Because an association with both the behavioural and cognitive measures was a prerequisite for a cluster to be selected for the mediation analysis, we subsequently tested whether clusters detected with either of the two measures were also related to the other measure. The results of these retention analyses are shown in Table 7.2 and 7.3. Five identified clusters were retained and added to the mediation analyses.

TABLE 7.2. **CLUSTER-WISE REGRESSION ANALYSES OF THE ASSOCIATION BETWEEN CBCL ADHP SCORE, NEPSY-II-NL ATT/EF SCORE AND CORTICAL THICKNESS**

DETECTION				RETENTION			
CBCL ADHP & CORTICAL THICKNESS				NEPSY-II-NL ATT/EF & CORTICAL THICKNESS CLUSTERS IDENTIFIED WITH CBCL ADHP			RETAINED FOR MEDIATION ANALYSIS[b]
CLUSTER	B (95% CI)	β	P[a]	B (95% CI)	β	P[b]	
LEFT HEMISPHERE							
Caudalmiddlefrontal	-0.04 (-0.06;-0.02)	-0.14	<0.001	0.04 (-0.01;0.10)	0.06	0.12	no
RIGHT HEMISPHERE							
Postcentral	-0.05 (-0.06;-0.03)	-0.22	<0.001	0.02 (-0.02;0.06)	0.04	0.34	no
Lateraloccipital	-0.05 (-0.07;-0.03)	-0.19	<0.001	0.05 (-0.01;0.10)	0.06	0.09	yes
Superiortemporal	-0.05 (-0.07;-0.03)	-0.16	<0.001	0.01 (-0.05;0.07)	0.02	0.64	no
Cuneus	-0.04 (-0.06;-0.02)	-0.15	<0.001	0.00 (-0.05;0.05)	0.00	0.96	no

NEPSY-II-NL ATT/EF & CORTICAL THICKNESS				CBCL ADHP & CORTICAL THICKNESS CLUSTERS IDENTIFIED WITH NEPSY-II-NL ATT/EF			RETAINED FOR MEDIATION ANALYSIS[b]
CLUSTER	B (95% CI)	β	P[a]	B (95% CI)	β	P[b]	
LEFT HEMISPHERE							
none found	-	-	-	N/A	N/A	N/A	N/A
RIGHT HEMISPHERE							
none found	-	-	-	N/A	N/A	N/A	N/A

NOTE. CBCL = Child Behavior Checklist; ADHP = attention-deficit/hyperactivity problems; NEPSY = neuropsychological assessment; ATT/EF = Attention and Executive Functioning. The CBCL ADHP and NEPSY ATT/EF scores were square root transformed, therefore B's are not interpretable. Both determinant (CBCL/NEPSY) and outcome (thickness) were residualized for age during assessment/scanning. Analyses adjusted for child gender, ethnicity, gestational age at birth, birth weight, psychostimulant use, IQ, maternal education, drinking during pregnancy, smoking during pregnancy and household income. A higher CBCL ADHP score indicates more attention and hyperactivity problems, a higher NEPSY score indicates better functioning. [a] To identify clusters in the discovery phase, the α-level was set to 0.05. [b] To define clusters as consistent and select them for the mediation analysis, the α-level was set to 0.1 (association at trend level or significant).

TABLE 7.3. **CLUSTER-WISE REGRESSION ANALYSES OF THE ASSOCIATION BETWEEN CBCL ADHP SCORE, NEPSY-II-NL ATT/EF SCORE AND GYRIFICATION**

DETECTION				RETENTION			
CBCL ADHP & GYRIFICATION				NEPSY-II-NL ATT/EF & GYRIFICATION CLUSTERS IDENTIFIED WITH CBCL ADHP			RETAINED FOR MEDIATION ANALYSIS[b]
CLUSTER	B (95% CI)	β	P[a]	B (95% CI)	β	P[b]	
LEFT HEMISPHERE							
Frontal/temporal/parietal (LH1)	-0.03 (-0.05;-0.02)	-0.14	<0.001	0.02 (-0.02;0.07)	0.04	0.29	no
Superiorparietal/postcentral (LH2)	-0.04 (-0.06;-0.01)	-0.11	0.006	0.03 (-0.03;0.10)	0.04	0.32	no
RIGHT HEMISPHERE							
Frontal/temporal/parietal (RH1)	-0.03 (-0.05;-0.02)	-0.13	0.001	0.04 (-0.01;0.10)	0.06	0.12	no
NEPSY-II-NL ATT/EF & GYRIFICATION				CBCL ADHP & GYRIFICATION CLUSTERS IDENTIFIED WITH NEPSY-II-NL ATT/EF			RETAINED FOR MEDIATION ANALYSIS[b]
CLUSTER	B (95% CI)	β	P[a]	B (95% CI)	β	P[b]	
LEFT HEMISPHERE							
Inferiorparietal (LH3)	0.06 (0.01;0.12)	0.08	0.03	-0.02 (-0.04;0.01)	-0.06	0.13	no
Frontal (LH4)	0.05 (0.01;0.09)	0.09	0.02	-0.02 (-0.04;-0.01)	-0.10	0.007	yes
RIGHT HEMISPHERE							
Frontal/parietal (RH2)	0.09 (0.03;0.15)	0.11	0.004	-0.02 (-0.05;0.00)	-0.07	0.07	yes
Frontal/temporal (RH3)	0.10 (-0.01;0.21)	0.07	0.07	-0.04 (-0.08;0.00)	-0.07	0.05	yes
Occipital (RH4)	0.06 (0.01;0.12)	0.08	0.02	-0.02 (-0.04;0.00)	-0.08	0.05	yes

NOTE. CBCL = Child Behavior Checklist; ADHP = attention-deficit/hyperactivity problems; NEPSY = neuropsychological assessment; ATT/EF = Attention and Executive Functioning. The CBCL ADHP and NEPSY ATT/EF scores were square root transformed, therefore B's are not interpretable. Both determinant (CBCL/NEPSY) and outcome (gyrification) were residualized for age during assessment/scanning. Analyses adjusted for child gender, ethnicity, gestational age at birth, birth weight, psychostimulant use, IQ, maternal education, drinking during pregnancy, smoking during pregnancy and household income. A higher CBCL ADHP score indicates more attention and hyperactivity problems, a higher NEPSY score indicates better functioning. [a] To identify clusters in the discovery phase, the α-level was set to 0.05. [b] To define clusters as consistent and select them for the mediation analysis, the α-level was set to 0.1 (association at trend level or significant).

Interrelation cognitive functioning, cortical morphology and ADHD problems

Lastly, we investigated whether the association between attention-deficit/hyperactivity problems and cognitive functioning in the domain Attention and Executive Functioning could be explained by the cortical morphology of the identified clusters. Therefore we performed a multiple mediation analysis, using as mediators only those clusters that were retained in the previous step.

The mediation analysis showed that both the direct effect of CBCL attention-deficit/hyperactivity problems on NEPSY-II-NL Attention and Executive Functioning (B=-0.033, p=0.02) and the total indirect effect through the selected cortical clusters (B=-0.008, bias-corrected 95% CI's ranging from -0.0172 to -0.0001) were statistically significant. This implies that the association between attention-deficit/hyperactivity problems and cognitive functioning in the domain Attention and Executive Functioning (B=-0.041, p=0.004) was at least partially explained by cortical morphology.

DISCUSSION

The aim of this study was to investigate the role of cortical morphology in the association between attention-deficit/hyperactivity symptoms and cognitive functioning. As hypothesized, we found that attention-deficit/hyperactivity problems were not related to cognitive functioning in general, but specifically to functioning in the domain of attention and EF. This finding is in line with previous clinical studies, that also showed deficits in EF to be most strongly related to ADHD (Frazier et al., 2004; Willcutt et al., 2005). These findings are consistent with one of the most influential theories of ADHD, suggesting that the symptoms and cognitive problems within ADHD actually result from a core deficit in inhibition (Barkley, 1997). Possibly, the weaker general cognitive functioning in previous ADHD studies (Frazier et al., 2004; Kuntsi et al., 2004) is partly driven by deficits in EF. Since the tasks in our study were designed to measure specific cognitive functions, with minimized interference of other functions, the specificity of EF problems in attention-deficit/hyperactivity problems could be tested. Another potential explanation could be that these previous studies were performed in clinical populations, where symptoms are usually more severe and where there is a higher chance of referral bias by impaired children.

In line with previous clinical studies (Castellanos & Proal, 2012; Leech & Sharp, 2014; Rubia, Alegria, & Brinson, 2014) we showed attention-deficit/hyperactivity problems to be associated with a thinner cortex over all four lobes of the brain. Similarly, we found less gyrification throughout large areas of the brain. Finally, we found similarly located clusters of less gyrification in children that performed worse on neuropsychological tasks measuring attention and EF.

Because of the shared genetic etiology of cognitive ability and ADHD (Kuntsi et al., 2004; Polderman et al., 2009) which suggests a common underlying neurobiology of attention-deficit/hyperactivity problems and cognition, and based on previous neuroimaging findings in ADHD (Mous, Karatekin, et al., 2014; Narr et al., 2009; Shaw et al., 2007; Wolosin et al., 2009), we hypothesized that cortical morphology could be the shared substrate underlying attention-deficit/hyperactivity problems and cognitive problems. Our results show that a shared cortical morphology indeed partly explained the association between attention-deficit/hyperactivity problems and EF. This finding enhances our understanding of the underlying neurobiology of attention-deficit/hyperactivity problems and co-occurring EF problems. Potentially, future studies investigating other imaging modalities (e.g connectivity)

may provide additional knowledge with regard to the shared neurobiology underlying these two constructs. Based on our results, it can be concluded that attention-deficit/hyperactivity and EF problems are at least partly explained by similar cortical abnormalities, indicating that the EF problems in ADHD should not be seen as a separate comorbid cognitive problem, but as part of the disorder. The cortical abnormalities that we found are nonspecific and cover large parts of the cortex, which suggests that both attention-deficit/hyperactivity problems and problems in EF are related to global deviations in cortical morphology. This is in line with previous clinical studies that have shown widespread cortical abnormalities (Narr et al., 2009; Shaw et al., 2007; Wolosin et al., 2009).

A limitation of our study is that the neuroimaging and neuropsychological data were collected at only one time point, therefore no inferences on causality or direction of effect can be made. Although we chose to perform a mediation analysis to formally test the role of cortical morphology in the relation between attention-deficit/hyperactivity problems and EF, we did not assume a causal pathway and our study does not draw any conclusions regarding the directionality in the associations studied. This crucial information, whether behavioral problems precede cognitive problems or vice versa, and how exactly cortical morphology is involved, remains to be elucidated. Longitudinal studies are needed to clarify this temporal direction. Also, the CBCL data was collected at a slightly earlier time point than the neuroimaging and neuropsychological data (mean time interval 1.9 years) and, although the CBCL attention-deficit/hyperactivity problems scores have been shown to have high stability over time in both clinical and population-based samples (Biederman et al., 2001; Verhulst & van der Ende, 1992), this may have influenced the results. However, given that associations remain despite a lag between measurements suggests a highly robust finding.

An important strength of the current study is that it is novel in the sense that no previous studies have assessed the role of cortical morphology in the association between attention-deficit/hyperactivity problems and cognitive functioning. Studying this topic helps us to understand the underlying neurobiology and high comorbidity of attention-deficit/hyperactivity problems and cognitive problems. In addition, the relationship between gyrification and attention-deficit/hyperactivity symptoms has never been tested in a non-clinical population. The population-based nature, as well as the large sample size, are important strengths of our study. By using a continuous problemscore, our study covers the entire spectrum of attention-deficit/hyperactivity problems, and thus includes both children with no or very few problems, as well as children with clinical problems. This provides greater generalizability with the general population compared to a study sample recruited from a clinical setting. Furthermore, we were able to correct for the use of psychostimulant medication. As psychostimulant use may alter brain structure (Rubia et al., 2014) and influences cognitive functioning (Coghill et al., 2013; Linssen, Sambeth, Vuurman, & Riedel, 2014), this is a very important potentially confounding factor.

To conclude, in a large population-based sample of school-aged children we found cortical thickness and gyrification to be related to attention-deficit/hyperactivity problems and EF and to partly explain the association between these two constructs. This suggests that cortical morphology is a shared neurobiological substrate underlying attention-deficit/hyperactivity problems and EF.

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SUPPLEMENT

TABLE S7.1. **PEARSON CORRELATIONS NEPSY-II-NL SUBTEST SCORES AND CORRESPONDING DOMAINSCORE (n=1,307)**

	TOTAL SCORE	ATTENTION AND EXECUTIVE FUNCTIONING SCORE	LANGUAGE SCORE	MEMORY AND LEARNING SCORE	SENSORIMOTOR FUNCTIONING SCORE	VISUOSPATIAL PROCESSING SCORE
Auditory Attention						
• Total score	0.55**	0.61**	n/a	n/a	n/a	n/a
• Commission errors	-0.37**	-0.43**	n/a	n/a	n/a	n/a
• Omission errors	-0.55**	-0.61**	n/a	n/a	n/a	n/a
• Inhibition errors	-0.24**	-0.33**	n/a	n/a	n/a	n/a
Response Set						
• Total score	0.76**	0.80**	n/a	n/a	n/a	n/a
• Commission errors	-0.66**	-0.72**	n/a	n/a	n/a	n/a
• Omission errors	-0.76**	-0.80**	n/a	n/a	n/a	n/a
• Inhibition errors	-0.58**	-0.63**	n/a	n/a	n/a	n/a
Statue						
• Total score	0.46**	0.57**	n/a	n/a	n/a	n/a
• Total movements	-0.41**	-0.49**	n/a	n/a	n/a	n/a
• Total sounds	-0.25**	-0.37**	n/a	n/a	n/a	n/a
• Total eye openings	-0.37**	-0.47**	n/a	n/a	n/a	n/a
Word Generation						
• Total of correct words Animals	0.52**	n/a	0.87**	n/a	n/a	n/a
• Total of correct words Foods/Drinks	0.53**	n/a	0.88**	n/a	n/a	n/a
Memory for Faces						
• Total score	0.36**	n/a	n/a	0.55**	n/a	n/a
Memory for Faces – delayed						
• Total score	0.35**	n/a	n/a	0.53**	n/a	n/a
Narrative Memory						
• Total score free and cued recall	0.62**	n/a	n/a	0.85**	n/a	n/a
• Total score free recall	0.58**	n/a	n/a	0.82**	n/a	n/a
• Total score recognition	0.45**	n/a	n/a	0.63**	n/a	n/a
Visuomotor Precision						
• Total time	0.04	n/a	n/a	n/a	-0.90**	n/a
• Total errors	-0.40**	n/a	n/a	n/a	0.68**	n/a
• Total pencil lifts	0.03	n/a	n/a	n/a	-0.79**	n/a
Arrows						
• Total score	0.51**	n/a	n/a	n/a	n/a	0.85**
Geometric Puzzles						
• Total score	0.29**	n/a	n/a	n/a	n/a	0.61**
Route Finding						
• Total score	0.56**	n/a	n/a	n/a	n/a	0.77**

NOTE. NEPSY = neuropsychological assessment. ** $p<0.01$.

TABLE S7.2. **ASSOCIATION BETWEEN CBCL ADHP SCORE[a] AND NEPSY-II-NL TOTAL- AND DOMAINSCORES**

	TOTAL SCORE[a]			ATTENTION AND EXECUTIVE FUNCTIONING DOMAIN SCORE[a]			LANGUAGE DOMAIN SCORE		
	B (95% CI)	β	P	B (95% CI)	β	P	B (95% CI)	β	P
Model I	-0.02 (-0.04;0.01)	-0.05	0.20	-0.04 (-0.07;-0.02)	-0.12	0.002**	0.05 (-0.04;0.14)	0.04	0.29
Model I + IQ	-0.01 (-0.03;0.02)	-0.03	0.48	-0.04 (-0.07;-0.01)	-0.11	0.004**	0.06 (-0.03;0.15)	0.05	0.19

	MEMORY AND LEARNING DOMAIN SCORE			SENSORIMOTOR FUNCTIONING DOMAIN SCORE			VISUOSPATIAL PROCESSING DOMAIN SCORE[a]		
	B (95% CI)	β	P	B (95% CI)	β	P	B (95% CI)	β	P
Model I	0.06 (-0.03;0.15)	0.05	0.17	-0.01 (-0.12;0.10)	-0.01	0.83	0.00 (-0.02;0.03)	0.01	0.84
Model I + IQ	0.08 (-0.01;0.17)	0.07	0.07	-0.03 (-0.13;0.08)	-0.02	0.63	0.02 (-0.01;0.04)	0.04	0.22

NOTE. CBCL = Child Behavior Checklist; ADHP = attention-deficit/hyperactivity problems; NEPSY = neuropsychological assessment. Both determinant (CBCL) and outcome (NEPSY) were residualized for age during assessment in all models. Model I was adjusted for child gender, ethnicity, gestational age at birth, birth weight, psychostimulant use, maternal education, drinking during pregnancy, smoking during pregnancy and household income. The B's are not interpretable since square root transformed scores ([a]) were used in the analyses. A higher CBCL ADHP score indicates more attention and hyperactivity problems, a higher NEPSY score indicates better functioning. **p<0.01

TABLE S7.3. **VERTEX-WISE ANALYSES OF CBCL ADHP SCORE AND NEPSY-II-NL ATT/EF SCORE WITH CORTICAL MORPHOLOGY**

	CLUSTER SIZE (MM^2)	TALAIRACH COORDINATES			NO. OF VERTICES WITHIN CLUSTER	CLUSTER-WISE (CORRECTED) P-VALUE
		TalX	TalY	TalZ		
CBCL ADHP & CORTICAL THICKNESS						
LEFT HEMISPHERE						
• Caudalmiddlefrontal	1049.32	-34.1	6.8	20.0	2039	0.009
RIGHT HEMISPHERE						
• Postcentral	6397.91	49.3	-13.2	47.9	15175	<0.001
• Lateraloccipital	1940.74	26.4	-91.9	13.1	2766	<0.001
• Superiortemporal	1470.95	48.1	-16.0	-7.8	3118	<0.001
• Cuneus	1677.35	11.5	-69.9	23.1	2529	<0.001
NEPSY-II-NL ATT/EF & CORTICAL THICKNESS						
LEFT HEMISPHERE						
• none	N/A	N/A	N/A	N/A	N/A	N/A
RIGHT HEMISPHERE						
• none	N/A	N/A	N/A	N/A	N/A	N/A
CBCL ADHP & GYRIFICATION						
LEFT HEMISPHERE						
• Frontal/temporal/parietal (LH1)	37822.11	-4.9	-62.3	26.6	74434	<0.001
• Superiorparietal/postcentral (LH2)	3903.37	-35.0	-29.7	61.0	9520	<0.001
RIGHT HEMISPHERE						
• Frontal/temporal/parietal (RH1)	36480.54	20.8	24.5	49.5	74882	<0.001
NEPSY-II-NL ATT/EF & GYRIFICATION						
LEFT HEMISPHERE						
• Inferiorparietal (LH3)	4359.38	-39.8	-77.9	19.2	8686	<0.001
• Frontal (LH4)	1933.25	-21.6	52.9	2.2	2880	0.04
RIGHT HEMISPHERE						
• Frontal/parietal (RH2)	12178.87	22.8	-4.6	44.1	26051	<0.001
• Frontal/temporal (RH3)	6222.21	44.2	35.6	-1.1	14233	<0.001
• Occipital (RH4)	2489.04	31.6	-77.6	-4.1	3333	0.01

NOTE. CBCL = Child Behavior Checklist; ADHP = attention-deficit/hyperactivity problems; NEPSY = neuropsychological assessment; ATT/EF = Attention and Executive Functioning. The CBCL and NEPSY scores were square root transformed. Analyses corrected for gender, age during scanning used as nuisance factor. Monte Carlo Simulation ($p<0.05$) was applied to correct for multiple testing. TalX = Talairach region X plane; TalY = Talairach region Y plane; TalZ = Talairach region Z plane.

CHAPTER

8 General discussion

This thesis focused on the neurobiology and neuropsychology of attention-deficit/hyperactivity problems. The majority of the studies described in this thesis were performed within the large population-based cohort study Generation R (Rotterdam, the Netherlands), in a sample of young children between six and nine years of age (Jaddoe et al., 2012; White et al., 2013). By using a population-based sample and continuous scores of inattention and hyperactivity symptoms, our study covers the entire spectrum of attention-deficit/hyperactivity problems, ranging from no problems to clinically significant problems. This offers the opportunity to extend previous findings based on clinical samples to the full range of problems in the general population. The aims of this thesis were 1) to explore the neurobiology (imaging and genetics) of attention-deficit/hyperactivity problems, 2) to study the normal development of cognitive ability, in order to 3) study cognitive problems associated with attention-deficit/hyperactivity problems. In this chapter, the main findings of this thesis are highlighted. Furthermore, methodological considerations are discussed and clinical implications, as well as suggestions for future studies, are addressed.

MAIN FINDINGS

Cortical morphology and attention-deficit/hyperactivity problems

Previous clinical studies have frequently shown abnormal cortical morphology in children with a diagnosis of attention-deficit/hyperactivity disorder (ADHD). Among findings that were reported, individuals with ADHD have been shown to have a thinner cortex, a delay in cortical maturation, and less gyrification (Narr et al., 2009; Shaw et al., 2007; Shaw et al., 2006; Shaw et al., 2012; Wolosin, Richardson, Hennessey, Denckla, & Mostofsky, 2009). Only two studies have previously evaluated the relationship between problems of inattention and hyperactivity and cortical thickness in non-clinical populations. These studies have also shown more severe problems to be related to a thinner cortex and a delay in maturation of the cortex (Ducharme et al., 2012; Shaw et al., 2011).

In this thesis, we studied the association between cortical morphology (thickness and gyrification) and inattention/hyperactivity symptoms along a continuum in a large population-based sample of young children (chapter 2 and 7). We showed that cortical thickness is related to symptoms of inattention and hyperactivity. In line with previous clinical studies, children with more symptoms of inattention and hyperactivity had thinner cortices in regions covering all lobes of the cortex. Similarly, we found attention-deficit/hyperactivity problems to be related to less gyrification throughout large areas of the frontal, temporal, parietal and cingulate cortices of the brain. The deviations in cortical thickness and gyrification that were observed were nonspecific, covering large parts of the cortex. This suggests that attention-deficit/hyperactivity problems are related to global deviations in cortical morphology. This is in line with previous clinical studies that have also shown widespread cortical abnormalities in children with ADHD(Narr et al., 2009; Shaw et al., 2007; Wolosin et al., 2009). In both chapter 2 and 7, the association between cortical thickness and inattention/hyperactivity symptoms was studied. In chapter 2 we only found an association with thickness in the somatosensory region of the brain, while in chapter 7 we found additional regions to be implicated. This difference in findings can most likely be attributed to power, as the study sample used in chapter 7 is nearly twice as large as the one described in chapter 2, allowing more vertices of the brain to pass the stringent multiple testing threshold.

In chapter 4, we compared patterns of brain gyrification between children with a clinical diagnosis of ADHD and children with velocardiofacial syndrome (VCFS). VCFS (also referred to as 22q11.2 deletion syndrome) is a genetic neurodevelopmental disorder characterized by palatal abnormalities, cardiac problems, and specific facial features. It is not uncommon for children with VCFS to show problems with attention, hyperactivity, and impulsivity (Shprintzen, 2000). Consequently, these children are often diagnosed with ADHD (Antshel et al., 2007). Despite the similarity of the inattention and hyperactivity problems, it is unclear if there is overlap in the neurobiology underlying VCFS and ADHD. In our study, we found only minor deviations in gyrification when we compared children with ADHD to normal controls. In children with VCFS we found larger and different abnormalities, showing a global decrease in gyrification. We did not find evidence for a common pattern of brain gyrification between ADHD and VCFS, although it must be noted that the sample size in our study was small (ADHD n=19, VCFS n=9, NC n=23).

Studies investigating typical cortical development in children have shown a characteristic temporal progression within regions of brain development. Over development, the primary sensorimotor cortices mature first, together with the frontal and occipital poles of the cortex. Maturation then progresses in a parietal to frontal wave of development (Gogtay et al., 2004). The development of cortical thickness follows an inverted u-shape. First, there is an increase in cortical thickness, and after the cortex reaches peak cortical thickness, the cortex starts thinning. Although the exact mechanisms are still unclear, it is thought that the initial increase in cortical thickness may be driven by mechanisms such as dendritic spine growth and the expansion of supporting glia (Chklovskii, Mel, & Svoboda, 2004; Sur & Rubenstein, 2005). The cortical thinning that follows may reflect intracortical myelination and the creation of efficient neural networks by the elimination of unused synapses (Hensch, 2004; Huttenlocher & Dabholkar, 1997). The thinner cortex that we found in our studies may thus suggest that the peak cortical thickness attained is less in children with more attention and hyperactivity problems. Alternatively, it may point to a deviation in the developmental trajectory of cortical thickness, which could either be earlier thinning, or a delay in reaching peak cortical thickness. A delayed maturation (and consequently later thinning) of the cortex has been found in a previous clinical longitudinal study (Shaw et al., 2007) and may point to less efficient brain networks, possibly causing the cognitive and behavioral difficulties which children with attention-deficit/hyperactivity problems experience. However, as the evidence for this theory of a maturational delay is limited and is restricted to clinical samples, additional longitudinal (population-based) studies with multiple measurement points are needed to shed light on the trajectories of cortical thickness in children with attention-deficit/hyperactivity problems.

Gyrification is the developmental process in which the brain forms the ridges (gyri) and grooves (sulci) that characterize a typical brain (Zilles, Armstrong, Schleicher, & Kretschmann, 1988). The process of gyrification begins between the 10th and 15th week of gestational life. However, most sulci and gyri form during the third trimester of fetal life, during a period of rapid brain growth (Chi, Dooling, & Gilles, 1977; Welker, 1990; White & Hilgetag, 2011; Zilles et al., 1997). Gyrification provides a greater surface area of the brain per unit volume and thus facilitates efficient packing of gray matter (Hilgetag & Barbas, 2006). This efficient packing of neurons likely implies a greater potential for computational abilities in the brain (Van Essen, 1997). The differences in gyrification that we found in our studies may thus suggest an altered development of gyrification and could reflect less efficient neuronal connectivity between brain regions. Less efficient connectivity could translate into less efficient neuronal processing, thereby resulting in the cognitive and behavioral problems that characterize ADHD.

Basal ganglia and attention-deficit/hyperactivity problems

The subcortical morphology of clinical ADHD has also been studied previously (Durston, 2003). Among findings, the most pronounced structural abnormalities were located in the structures comprising the basal ganglia. In previous studies, a reduction of volume of these structures has been found consistently in patients with ADHD (Frodl & Skokauskas, 2012; Nakao, Radua, Rubia, & Mataix-Cols, 2011; Shaw et al., 2014).

In chapter 3 of this thesis, we studied the association between symptoms of inattention and hyperactivity/impulsivity (along a continuum) and volume of three basal ganglia structures (putamen, pallidum and caudate) in a large population-based sample of young children. In line with the previous clinical studies, we demonstrated that children with more inattention and hyperactivity/impulsivity problems had smaller volumes of the putamen.

As part of the fronto-striatal circuitry, the basal ganglia are modulated by different neurotransmitter systems, including the dopaminergic, serotonergic and noradrenergic systems (Carli & Invernizzi, 2014; Di Matteo et al., 2008). An imbalance in these neurotransmitter systems may thus be related to the changes in basal ganglia structure as reported in our and previous studies. Similarly, higher dopamine transporter density and, consequently, a reduced availability of dopamine in the basal ganglia have been reported in patients with ADHD (Cheon et al., 2003; Dougherty et al., 1999; Larisch et al., 2006; Volkow et al., 2007). Accordingly, methylphenidate, the most frequently used drug in alleviating symptoms of ADHD, acts upon the dopamine system by blocking dopamine transporter levels and increasing the availability of dopamine in the basal ganglia. Thereby, the hypodopaminergic fronto-striatal circuitry gets upregulated, thereby reducing the symptoms of inattention and hyperactivity (Rubia et al., 2009; Volkow et al., 2007). In previous (molecular) genetic studies, the dopaminergic system has frequently been discussed as potential causal pathway in ADHD (Caylak, 2012; Cortese, 2012; Faraone, Bonvicini, & Scassellati, 2014; Volkow et al., 2007) and our and previous findings of the involvement of the basal ganglia in attention-deficit/hyperactivity disorder indirectly provides additional support for a role of this neurotransmitter system in ADHD. With regard to the functions of the basal ganglia, most research has focused on the known role in motor behaviour. However, other studies have also shown the basal ganglia (and specifically the putamen) to be involved in (somato)sensory, affective, working memory and other higher order executive functioning processes (Arsalidou, Duerden, & Taylor, 2013), functions that are typically impaired in ADHD. Based on the findings of our neuroimaging study we can conclude that there is support for a role of the putamen and (indirectly) of the dopaminergic system in the neurobiology of attention-deficit/hyperactivity symptoms.

Cognition and attention-deficit/hyperactivity problems

In addition to the behavioral problems that characterize ADHD, cognitive problems are commonly found in children with attention-deficit/hyperactivity problems. Studies have shown moderate correlations between ADHD symptom scores and IQ scores and a significantly lower mean IQ in children with ADHD (Frazier, Demaree, & Youngstrom, 2004; Kuntsi et al., 2004).

In order to better understand problems in cognitive development, it is important to understand the typical development of cognitive ability in children. Therefore, in chapter 5 of this thesis, we studied the association of neuropsychological functioning with age, gender and intelligence in a large sample of typically developing children. An extensive neuropsychological assessment battery was performed,

assessing the domains attention and executive functioning, language, memory and learning, sensorimotor functioning, and visuospatial processing. As expected, we found strong associations between age and functioning in all domains, showing that older children performed better on the tasks compared to younger children. For some tasks, we found that performance remained relatively stable from a certain age range onwards, suggesting that the children were reaching mastery within the age range of our study (6 to 10 years of age). In addition to the expected age-related differences, clear gender differences were found, showing that girls generally outperformed boys, with the exception of visuospatial tasks. Furthermore, IQ was positively associated with neuropsychological functioning, which was strongest in visuospatial tasks.

In addition to expanding our understanding of normal cognitive development, it is also desirable to better grasp potential neurobiological underpinnings of cognitive functioning. One feature of the brain that may underlie cognitive functioning is structural connectivity. For cognitive functioning, efficient communication between different interacting brain regions is highly important. Therefore, previous studies have studied white matter integrity in relation to cognitive ability (Erus et al., 2015; Fryer et al., 2008; Johansen-Berg, Della-Maggiore, Behrens, Smith, & Paus, 2007; Muetzel et al., 2008; Navas-Sanchez et al., 2014; Schmithorst, Wilke, Dardzinski, & Holland, 2002) and have found white matter integrity to be positively related to cognitive functioning. Because studies in large general population samples of young children are lacking, and because little is known with regard to different general domains of cognitive functioning, we studied the association between white matter integrity and neuropsychological functioning in chapter 6 of this thesis. We demonstrated a positive association between white matter integrity and general cognitive functioning (as represented by non-verbal IQ), independent of age. Furthermore, of the specific neuropsychological domains that were studied, we found visuospatial ability to be positively associated with white matter integrity. Our findings thus confirm a role of structural connectivity in cognitive functioning, independently of age or maturation of the brain.

As white matter development, such as myelination, plays an important role in modulating diffusion (an)isotropy, our finding of better cognitive functioning in relation to white matter integrity may be partly explained by higher levels of myelination. Since the process of myelination leads to a faster communication between more distant regions of the brain, this may result in more efficient interconnectivity of these regions. This more efficient connectivity most likely enhances the ability to utilize and manipulate information in the brain and consequently facilitates cognitive functioning. The fact that we only found an association between white matter integrity and visuospatial ability when assessing the specific neuropsychological functions can possibly be explained by residual age-effects. Since most cognitive functions are still developing in such young children, individual maturational differences may have masked the (weaker) associations between these other cognitive functions and white matter integrity.

As mentioned earlier, ADHD has been related to worse cognitive functioning. However, it is unclear whether these deficits in cognitive functioning represent a general cognitive deficit, or actually reflect deficits in more specific cognitive domains. Although previous clinical studies have suggested a wide range of neuropsychological domains to be affected in patients with ADHD (Brodsky, Willcutt, Davalos, & Ross, 2014; Frazier et al., 2004; Willcutt, Sonuga-Barke, Nigg, & Sergeant, 2008), two large meta-analyses (Frazier et al., 2004; Willcutt et al., 2008) have shown that ADHD seems to be most strongly asso-

ciated with problems in executive functioning. In chapter 7 of this thesis, we studied the association between neuropsychological functioning and symptoms of inattention and hyperactivity in a large population-based sample of young children. In accordance with the previous clinical studies, we found that children with more attention-deficit/hyperactivity problems show specific problems in executive functioning, rather than a global deficit in cognitive functioning. This finding is in line with one of the most influential theories of ADHD, suggesting that the symptoms and cognitive problems within ADHD actually result from a core deficit in inhibition (Barkley, 1997). Possibly, the weaker general cognitive functioning that was found in previous ADHD studies (Frazier et al., 2004; Kuntsi et al., 2004) is mainly driven or caused by deficits in executive functioning. As processes such as paying attention, keeping instructions active in working memory, shifting between functions or tasks, and inhibiting unnecessary responses are key elements to successful learning and cognitive functioning, problems in these executive functions may have resulted in worse general cognitive functioning and lower IQ scores. Alternatively, it might be that the initial executive functioning problems give rise to problems in general cognitive functioning as children get older. One could envision that long-term attention, inhibition and (working) memory problems may result in a reduced learning potential and a sub-optimal learning circumstance at school, possibly leading to an educational delay and learning difficulties. Longitudinal studies are needed to address this question.

Cortical morphology as shared neurobiological substrate underlying inattention/hyperactivity symptoms and cognitive problems

Based on the frequent comorbidity of attention-deficit/hyperactivity problems and cognitive problems, we studied the underlying neurobiology of these two constructs in chapter 7 of this thesis. Previous twin studies have revealed a shared genetic etiology of cognitive ability and ADHD (Kuntsi et al., 2004; Polderman et al., 2009; Polderman et al., 2006). This shared genetic background suggests that a common neurobiology underlies both ADHD and cognition. Based on previous neuroimaging findings in ADHD (Narr et al., 2009; Shaw et al., 2007; Wolosin et al., 2009), one might expect that this shared neurobiology could be reflected in cortical morphology. Therefore, we studied cortical morphology as potential shared neurobiological substrate underlying both executive functioning problems and symptoms of inattention and hyperactivity. We found that thickness and gyrification of different areas of the cortex partly explained the association between executive functioning problems and symptoms of inattention and hyperactivity, indeed pointing towards a shared neurobiology of the two constructs, partly reflected in cortical morphology.

The findings of this study enhance our understanding of the neurobiology of attention-deficit/hyperactivity problems and co-occurring executive functioning problems. Furthermore, the results of our study indicate that the executive functioning problems in ADHD should not be seen as a separate comorbid cognitive problem, but should rather be regarded part of the disorder.

Candidate genetic pathways and attention-deficit/hyperactivity problems

Although ADHD is one of the most common neurodevelopmental disorders, little is known about the genetics underlying the disorder. Recent studies have shown high heritability estimates of around 70% (Faraone et al., 2005; Nikolas & Burt, 2010; Posthuma & Polderman, 2013), but despite this, discovering genes that are associated with ADHD has proven to be difficult. Because ADHD is highly heter-

ogeneous and polygenic (implying that many genes, each having a very small effect, are involved), genome-wide association studies (GWAS) have not been successful in identifying responsible genes (Neale et al., 2010). To overcome the problems associated with the polygenic character of ADHD, new approaches have been sought, including gene-set analyses. In gene-set analyses, single genes are combined into (functional) gene-sets, thereby decreasing multiple testing and increasing power (Lips et al., 2012; Wang, Li, & Bucan, 2007).

A previous study in a clinical sample of children with ADHD (Bralten et al., 2013) has shown that gene-sets involved in dopamine/norepinephrine and serotonin neurotransmission and neuritic outgrowth are associated with hyperactivity/impulsivity symptom severity. To assess whether this finding can be replicated in and generalized to the general population, we studied the association between these gene-sets and symptoms of inattention and hyperactivity/impulsivity in a large population-based sample of children in chapter 3 of this thesis. Although gene-sets that are defined by their involvement in a specific neurotransmitter pathway that is presumed to be implicated in the phenotype of interest probably have a higher prior probability of association with the phenotype compared to discovery GWAS, the prior probability most likely remains to be relatively low in a multifactorial and complex disorder such as ADHD (Colhoun, McKeigue, & Davey Smith, 2003). Based on a previous study, this implies that empirical p-values should be 1x10-5 or smaller to yield a high positive predictive value (PPV) and thus indicate a high probability of true association (Broer et al., 2013). In order to have enough power to reach this level of statistical significance, a large sample size combined with a substantial effect is needed.

In our study, we did not find support for a role of the selected dopamine/norepinephrine, serotonin and neuritic outgrowth gene-sets in inattention or hyperactivity/impulsivity symptom severity. Given the large sample size of our study (n=1,871), this implies that the effect, if present at all, is most likely to be very small and might necessitate even larger sample sizes. Although our study was performed in a general population sample, which is less severely affected compared to a clinical population (such as the previous study using these gene-sets), our sample did include children that had clinically elevated scores (8% for inattention and 6% for hyperactivity/impulsivity symptom scores). However, if effects are indeed small and difficult to detect, our (although large) population-based sample with a small percentage of severely affected individuals may potentially have lacked the power to detect these small effects.

METHODOLOGICAL CONSIDERATIONS

Categorical versus dimensional approaches

In the last years, concurrently with the development of the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) (American Psychiatric Association, 2013), a debate has started whether child psychopathology (such as ADHD) might be better described within a dimensional framework, rather than with clearly defined diagnostic categories. Within this framework of continuous symptom levels, children with clinical disorders constitute the extreme end of the spectrum. Numerous studies demonstrate that such a dimensional approach can further contribute to a better etiological understanding of child psychopathology (Hudziak, Achenbach, Althoff, & Pine, 2007; Lubke, Hudziak,

Derks, van Bijsterveldt, & Boomsma, 2009; Polderman et al., 2007; Shaw et al., 2011) and heritability estimates of ADHD symptoms along a continuum have been shown to be similar to that of the categorically defined disorder (Hudziak, Rudiger, Neale, Heath, & Todd, 2000; Levy, Hay, McStephen, Wood, & Waldman, 1997; Polderman et al., 2007; Rietveld, Hudziak, Bartels, van Beijsterveldt, & Boomsma, 2004; Thapar, Harrington, Ross, & McGuffin, 2000). The findings described in this thesis, which were obtained in a sample from the general population, also provide support for the dimensionality of attention-deficit/hyperactivity problems.

Currently, the dimensionality of psychopathology has been cautiously introduced in the DSM-5 by the addition of a continuous measure indicating the degree of severity on top of the still existing categorical approach indicating the presence or absence of a psychiatric diagnosis. Although this way of combining categorical and dimensional approaches is somewhat basic and could potentially be better integrated, the two forms of information do complement each other, which might benefit both clinical practice and research. Clinicians still have the clear categories that they need to be able to make a decision on whether or not to treat a patient, but in addition to this, the indication of severity within the diagnosis may help them in developing a more personally directed treatment plan and to assess treatment success (Hudziak et al., 2007; Kraemer, 2007; Lopez, Compton, Grant, & Breiling, 2007). However, with the current implementation of the severity measure in the DSM-5, the presence of a diagnosis is a prerequisite in order to obtain a severity score. This means that children who fall just below the number of criteria necessary for a diagnosis of ADHD, but who do show a lot of symptoms that might negatively affect their lives, are not regarded. Since previous studies have clearly proved the dimensionality of and variation in the expression of symptoms, as well as the effect of age, gender and informant, clinical practice will in the future hopefully benefit even more from dimensional measures as they might be a more naturalistic representation of child psychopathology (Hudziak et al., 2007). This might for example be achieved by obtaining a truly continuous (symptom) score for every child admitted to a child psychiatric clinic, regardless of the eventual (categorical) diagnosis. In order to make diagnostic decisions on whether or not to treat a patient, cut points could be applied. For research purposes such a continuous score would also be favorable, as researchers gain the statistical power of continuous measures. Finally, by combining such dimensional information with the categorical diagnostic information, researchers will also still have the opportunity to select eligible participants for their study based on categorical diagnoses if desired (Hudziak et al., 2007; Kraemer, 2007; Lopez et al., 2007). In this way, the combination of categorical and dimensional information will eventually aid both clinical practice and research.

Whole brain versus Region of Interest imaging analysis

Two different approaches in neuroimaging analysis are whole brain and region of interest (ROI) techniques. ROI-based analysis is a technique that requires the identification of regions of interest and restricts the analyses to these specific regions. On the contrary, whole brain analysis covers all vertices of the brain and is not bound to a priori-defined regions or hypotheses.

Since findings with regard to cortical abnormalities in ADHD have been mixed, and since the boundaries of a priori-defined regions of interest may not exactly overlap the boundaries of the actual areas in which abnormalities are located, we chose to perform vertex-wise exploratory analyses of cortical morphology across the entire brain in chapters 2 and 7 of this thesis. An important drawback of this

hypothesis-free approach is that it can lead to data dredging, as the association of all vertices in the brain can be tested against any phenotype of interest. Additionally, as all vertices are tested against the phenotype, a stringent correction for multiple testing is required to avoid false positive results. Therefore, large sample sizes, such as the Generation R cohort, are needed to successfully employ this approach. For smaller studies, ROI-based approaches may therefore be more suitable. The main disadvantage of ROI analyses however, is that one needs to have a clear hypothesis on where abnormalities are most likely to be located, which is not always evident. In addition, since researchers usually rely on previously published findings for the selection of their ROIs, this approach is potentially more likely to be affected by publication bias.

Gene-sets versus single genes

As mentioned earlier, recent GWAS have not been very successful in the identification of genes that are associated with attention-deficit/hyperactivity problems (Neale et al., 2010). Because of the polygenic nature of ADHD, very large samples are needed to reach genome-wide significance. Likewise, candidate gene studies have identified some potentially related genes (Caylak, 2012), but replication of these findings has often proved to be challenging (Ioannidis, Trikalinos, Ntzani, & Contopoulos-Ioannidis, 2003). Furthermore, interpretation of a finding regarding a single gene or Single Nucleotide Polymorphism (SNP) may be difficult.

Because of these challenges, new approaches are being developed that account for the polygenic nature of complex psychiatric disorders such as ADHD. In chapter 3 of this thesis, we used gene-set analyses to test the association between candidate genetic pathways and symptoms of inattention and hyperactivity/impulsivity. In gene-set analyses, single genes are combined in gene-sets (for example based on their cellular function or involvement in a certain neurotransmitter pathway) and are jointly tested for association with the phenotype of interest (Lips et al., 2012; Wang et al., 2007). Gene-set analyses generally show increased power, as they suffer less from multiple testing compared to testing multiple separate genes or SNPs, and therefore smaller sample sizes will most likely suffice. In addition, using gene-sets that contain genes with a similar cellular function provides a more direct route to knowledge about the underlying biological mechanism of the disorder. Furthermore, findings may be easier to interpret compared to interpreting the effect of a single gene or SNP. There are also some drawbacks regarding the use of gene-set analyses, including the issue that the creation of such gene-sets requires a priori knowledge about the function of genes or SNPs, which is not always evident. Furthermore, SNPs that fall outside genes are generally poorly represented and are, as a consequence, rarely tested for association. Finally, as different ways of combining genes in sets can be employed, the use of differently formed gene-sets might lead to data dredging. Therefore, for future studies it is of great importance to further invest in, and make use of, empirically derived (standard) gene-sets covering the entire genome.

CLINICAL IMPLICATIONS

With cross-sectional observational studies, such as the studies described in this thesis, it is difficult to identify cause-effect relationships. However, our studies may shed new light on the biological processes underlying attention-deficit/hyperactivity problems and may identify important targets for (non-) pharmacological treatments and interventions.

In the last years, neuroimaging has advanced greatly and has started to play a critical role in psychiatry. Nowadays, neuroimaging features lack the desired sensitivity and positive predictive value to assist in the diagnostic process of complex developmental psychiatric disorders such as ADHD. However, the field is advancing at a high rate. Currently, pioneers in the field are studying machine-learning approaches that, in the future, may be able to classify children with attention-deficit/hyperactivity problems, based on multiple imaging modalities (Rubia, Alegria, & Brinson, 2014). In the future, this information may complement other (observational) diagnostic information and may assist psychiatrists in diagnostic decision-making.

In the meantime, neuroimaging does assist in finding biological pathways underlying disorders and can be used to identify disorder-specific biomarkers, which may eventually be useful for diagnostic and treatment decisions. As an example, studies comparing ADHD with other frequently co-occurring childhood disorders such as conduct disorder and oppositional defiant disorder have provided accumulating evidence for specific structural and functional abnormalities in the inferior frontal cortex, dorsolateral prefrontal cortex and the basal ganglia in ADHD, thereby serving as a putative disorder-specific biomarker of ADHD (Rubia et al., 2014). Neuroimaging features that are found to be specifically related to a certain disorder or trait, may potentially serve as useful intermediate phenotypes in investigating the genetics of that disorder or trait (White & Gottesman, 2012). Although successful applications of intermediate phenotypes in psychiatric gene finding are sparse, the approach has been successfully employed in a study on the genetics of alcoholism, and in studies investigating schizophrenia (Walters & Owen, 2007). These studies show that a cautious choice of a putative intermediate phenotype based on robust evidence may indeed assist in the discovery of disorder-related genes and may shed light on potential biological pathways. By providing insights into the biological processes implicated in psychiatric disorders such as ADHD, potential new (drug) targets can be identified, thereby aiding the development of new medications or other non-pharmacological treatments. Furthermore, neuroimaging features can be used to evaluate treatment success, by assessing changes/normalization in brain morphology or function over time (Linden, 2012).

The previous finding of a shared genetic background of cognitive ability and ADHD, complemented with our finding of cortical morphology as a shared neurobiological substrate underlying ADHD symptoms and executive functioning problems (chapter 7), indicates that the executive functioning problems in children with ADHD should not be seen as a separate comorbid cognitive problem, but should actually be regarded part of the disorder. In the current DSM-5 classification system (American Psychiatric Association, 2013), cognitive problems are not considered a criterion for the diagnosis of ADHD, but are rather seen as a comorbid problem. The results of our and previous studies argue that the presence of executive functioning problems should be considered essential information in diagnostic decision-making in ADHD. Based on our findings, executive functioning problems should be evaluated as standard part of the diagnostic process when there is a presumption of ADHD in a

child. By doing so, executive functioning problems can be recognized early and interventions (such as therapy specifically aiming at improving executive functions and self-control) can be implemented complementary to conventional pharmacological treatment. In this way we can help these children in achieving better executive functioning skills, potentially resulting in reduced ADHD symptomatology and a reduced risk for learning problems.

FUTURE RESEARCH

When looking into the future, more studies need to be initiated that combine different research fields, like neuroimaging and genetics. By combining different disciplines, a more detailed knowledge of the neurobiology of psychiatric disorders such as ADHD will be established, leading to better treatment and more focused interventions. Furthermore, more studies should be performed in general population samples, as this provides important additional insights into the neurobiology and neuropsychology of attention-deficit/hyperactivity problems over the entire spectrum of problems. Ideally, population-based samples of different age ranges (and thus in different stages of brain development) should be studied. Most importantly, longitudinal population-based studies should be initiated. By doing so, trajectories of and changes in brain development or cognitive development can be studied thoroughly and cause-effect relationships can be identified.

Within neuroimaging research, the combination of different imaging modalities should be applied more often, as this may yield new discoveries and may eventually assist psychiatrists in classifying children with ADHD by providing additional medical information on top of the already available observational data. Within genetic studies of complex disorders like ADHD, new approaches such as gene-set analyses need to be further expanded and further developed. Future studies should consider focusing on gene-sets that are empirically derived and are defined by a shared cellular function, to gain more knowledge on the neurobiology of ADHD on a basic (cellular) level.

Lastly, most (neurobiological) research nowadays is focusing on children with a psychiatric diagnosis of ADHD or, incidentally, on parent-report of symptoms. Future studies should consider using other sources of information as well. As the behavior of the child may vary across setting, other informants (such as teachers or the child itself) may provide valuable additional information in creating a more complete picture of the behavioral and cognitive problems of children with attention-deficit/hyperactivity symptoms.

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Summary

Samenvatting

SUMMARY

Attention-deficit/hyperactivity disorder (ADHD) is one of the most common neurodevelopmental disorders, with a worldwide prevalence of about 3-5%. Over the last years, the notion that child psychopathology, such as ADHD, may be better described within a dimensional framework, has gained support. Within this framework of continuous symptom levels, the entire spectrum of problem behavior is covered, with children with clinical disorders constituting the extreme end of the spectrum. Although attention-deficit/hyperactivity problems have a high prevalence, relatively little is known with regard to the underlying neurobiology and especially studies in large general population samples are lacking. This thesis focused on the neurobiology and neuropsychology of attention-deficit/hyperactivity problems. The majority of the studies described in this thesis were performed in the Generation R Study, a large prospective population-based cohort study in Rotterdam, the Netherlands. In a large subsample of school-aged children, brain MRI scans were performed and data regarding attention-deficit/hyperactivity problems and cognitive functioning were gathered. The aims of this thesis were 1) to explore the neurobiology (imaging and genetics) of attention-deficit/hyperactivity problems, 2) to study the normal development of cognitive ability, in order to 3) study cognitive problems associated with attention-deficit/hyperactivity problems.

In chapter 2 we studied the association between cortical thickness and inattention/hyperactivity symptoms along a continuum in a large population-based sample of young children. We showed that cortical thickness is related to symptoms of inattention and hyperactivity. Children with more attention and hyperactivity problems had a thinner cortex in the somatosensory region of the brain. As the sensorimotor cortices are the first to reach maturation in normal development, it is not surprising that we found the effect in this region specifically. As other brain regions are still developing in these young children, it is possible that cortical thickness deviations in these regions will emerge later as the neurodevelopmental differences become unmasked. Our finding of a thinner cortex related to more attention-deficit/hyperactivity problems might imply that peak cortical thickness is less in children with more problems. Alternatively, it could point at a deviation in the developmental trajectory of cortical thickness in these children.

In chapter 3 we used a combination of neuroimaging and genetics to study the neurobiology of attention-deficit/hyperactivity problems in the general population. In this chapter we specifically studied the role of volume of basal ganglia structures and candidate genetic pathways (involved in dopamine/norepinephrine and serotonin neurotransmission and neuritic outgrowth) in relation to attention-deficit/hyperactivity problems. In line with clinical ADHD studies, we demonstrated an association between volume of the putamen and inattention and hyperactivity/impulsivity symptoms. Volume of this structure was smaller in children with more problems. Our study provides support for a role of the basal ganglia in attention-deficit/hyperactivity problems and shows that previously drawn conclusions based on clinical studies can be extended to the general population. Although successful in a previous clinical sample, we did not find support for a role of gene-sets involved in dopamine/norepinephrine and serotonin neurotransmission and neuritic outgrowth in our population-based sample. Our large study shows that, if any effect of these genetic pathways is present at all, it is most likely to be a very small effect. Further research is needed to investigate the role of these (and other) genetic pathways in attention-deficit/hyperactivity problems.

The study described in chapter 4 was performed in a different sample, namely a clinical population of children with ADHD, velocardiofacial syndrome (VCFS) and healthy controls. Since it is not uncommon for children with VCFS to show problems with attention, hyperactivity, and impulsivity, these children are often diagnosed with ADHD. Therefore, we compared patterns of brain gyrification between these groups of children. In spite of the similarities of the phenotypes in attention and hyperactivity problems, we found no evidence for a common pattern of brain gyrification between ADHD and VCFS. In children with ADHD we found only minor local deviations in gyrification, while in children with VCFS we found larger and different abnormalities, showing a global decrease in gyrification.

Attention-deficit/hyperactivity problems are commonly accompanied by cognitive problems. In order to understand the cognitive problems in child psychopathology, one should also be familiar with the typical development of cognitive ability in children. Therefore, in chapter 5, we studied the association of a broad range of neuropsychological functions with age, gender and intelligence in a large sample of typically developing children. As expected, we found strong effects of age, with older children performing better on all cognitive domains compared to younger children. For some functions, we found that performance remained relatively stable from a certain age range onwards, suggesting that the children were reaching mastery. In addition, clear gender differences were found, showing that girls generally outperformed boys, with the exception of visuospatial tasks. Finally, IQ was positively associated with neuropsychological functioning, which was strongest in visuospatial tasks.

As efficient communication between different brain regions is crucial for cognitive functioning, we examined the relation between white matter integrity and neuropsychological functioning in a large population-based sample of young children in chapter 6. We demonstrated a positive association of white matter integrity with general cognitive functioning (as represented by non-verbal IQ), as well as with visuospatial functions. Potentially, our finding of better cognitive functioning in relation to white matter integrity may be explained by higher levels of myelination and, as a result, more efficient interconnectivity and communication of brain regions.

In chapter 7, we studied whether children with attention-deficit/hyperactivity problems experience a general cognitive problem, or problems in specific cognitive functions. In addition, we examined cortical morphology (cortical thickness and gyrification) as potential shared neurobiological substrate underlying both the attention-deficit/hyperactivity problems and cognitive ability. In this chapter, we found that attention-deficit/hyperactivity problems in the general population are related to specific problems in executive functioning, rather than to a general cognitive problem. We also found that more attention-deficit/hyperactivity problems and worse functioning on executive functioning tasks was related to decreased cortical thickness and gyrification throughout multiple regions of the brain. Further, we demonstrated that cortical morphology partly explained the association between attention-deficit/hyperactivity problems and executive functioning, indeed implying that cortical morphology is a shared neurobiological substrate underlying these two constructs. This suggests that executive functioning problems in ADHD should not be seen as a separate comorbid cognitive problem, but should rather be regarded part of the disorder.

In the final part of this thesis, chapter 8, a general discussion of the main findings of the studies in this thesis is provided. Furthermore, this chapter describes major methodological considerations, as well as implications for clinical practice and future research.

SAMENVATTING

Aandachtstekort-hyperactiviteitstoornis (ADHD) is een van de meest voorkomende ontwikkelingsstoornissen, met een wereldwijde prevalentie van 3-5%. In de laatste jaren is het idee ontstaan dat psychopathologie bij kinderen beter op een dimensionele manier beschreven zou kunnen worden. Binnen deze dimensionele aanpak, waarin symptomen aan de hand van een continuüm beschreven worden, wordt het hele spectrum van probleemgedrag gedekt, waarbij kinderen met klinische problemen zich aan het extreme eind van het spectrum bevinden. Ondanks de hoge prevalentie van aandachts- en hyperactiviteitsproblemen is er relatief weinig bekend over de onderliggende neurobiologie, en ontbreken studies in grote populatie-gebaseerde steekproeven. Dit proefschrift richt zich op de neurobiologie en neuropsychologie van aandachts- en hyperactiviteitsproblemen. Het merendeel van de studies die beschreven worden in dit proefschrift zijn gedaan binnen de Generation R Studie, een grootschalige prospectieve populatie-gebaseerde cohort studie in Rotterdam. In een grote groep kinderen op basisschoolleeftijd zijn MRI scans van de hersenen gemaakt en zijn gegevens verzameld over aandachts- en hyperactiviteitsproblematiek en cognitief functioneren. Het doel van dit proefschrift was 1) het onderzoeken van de neurobiologie (beeldvorming van de hersenen en genetica) van aandachts- en hyperactiviteitsproblemen, 2) het bestuderen van de normale cognitieve ontwikkeling van kinderen, om vervolgens 3) de cognitieve problemen geassocieerd met aandachts- en hyperactiviteitsproblemen te onderzoeken.

In hoofdstuk 2 hebben we de relatie tussen de dikte van de cortex en aandachts- en hyperactiviteitsproblemen (over een continuüm) onderzocht in een grote populatie-gebaseerde steekproef van jonge kinderen. We hebben aangetoond dat corticale dikte gerelateerd is aan symptomen van onoplettendheid en hyperactiviteit. Kinderen met meer aandachts- en hyperactiviteitsproblemen hadden een dunnere somatosensorische cortex. Aangezien de sensorisch-motorische gebieden in de hersenen als eerste volgroeid raken in de normale ontwikkeling, is het niet verrassend dat wij het effect specifiek in dit gebied vonden. Omdat andere hersengebieden nog volop in ontwikkeling zijn, kan het zo zijn dat de associatie met corticale dikte later ook in deze gebieden zichtbaar zal worden. Onze bevinding van een dunnere cortex gerelateerd aan meer aandachts- en hyperactiviteitsproblemen kan er mogelijk op duiden dat de maximale corticale dikte kleiner is bij kinderen met meer problemen. Een alternatieve verklaring is dat er sprake is van een afwijking in het ontwikkelingstraject van corticale dikte.

In hoofdstuk 3 hebben we een combinatie van MRI en genetische analyses gebruikt om de neurobiologie van aandachts- en hyperactiviteitsproblemen te onderzoeken in de algemene populatie. In dit hoofdstuk hebben we ons specifiek gericht op de rol van het volume van de basale ganglia en kandidaat gen-sets (betrokken bij dopamine/norepinephrine en serotonine neurotransmissie en de groei van neurieten), in relatie tot aandachts- en hyperactiviteitsproblemen. In overeenstemming met klinische ADHD studies hebben we laten zien dat er een relatie bestaat tussen het volume van de putamen en aandachts- en hyperactiviteitsproblemen. Het volume van deze structuur was kleiner in kinderen met meer problemen. Onze studie ondersteunt daarmee de bevinding dat de basale ganglia een rol spelen in aandachts- en hyperactiviteitsproblemen en laat zien dat we eerder getrokken conclusies op basis van klinische studies kunnen doortrekken naar de algemene populatie. Ondanks een eerder aangetoonde relatie in een klinische populatie, vonden we geen aanwijzingen voor een rol van de gen-sets betrokken bij dopamine/norepinephrine en serotonine neurotransmissie en de groei van

neurieten in onze populatie-gebaseerde steekproef. Onze grote studie laat zien dat, als er werkelijk een effect van deze gen-sets bestaat, dit zeer waarschijnlijk een erg klein effect betreft. Er is meer onderzoek nodig naar de rol van deze (en andere) gen-sets binnen aandachts- en hyperactiviteitsproblemen.

De studie beschreven in hoofdstuk 4 is uitgevoerd in een andere studiepopulatie, namelijk een klinische populatie bestaande uit kinderen met ADHD, velocardiofaciaal syndroom (VCFS) en gezonde controles. Omdat veel kinderen met VCFS ook aandachts- en hyperactiviteitsproblemen vertonen, komt het vaak voor dat deze kinderen een diagnose ADHD krijgen. Daarom hebben we gyrificatie van de hersenen vergeleken tussen deze groepen kinderen. Ondanks de overeenkomsten in aandachts- en hyperactiviteitsproblematiek vonden we geen bewijs voor een vergelijkbaar patroon van gyrificatie van de hersenen bij kinderen met ADHD en VCFS. Bij kinderen met ADHD vonden we slechts kleine lokale afwijkingen in gyrificatie, terwijl we bij de kinderen met VCFS grotere en andere afwijkingen vonden, duidend op een globale vermindering van gyrificatie.

Aandachts- en hyperactiviteitsproblemen gaan vaak gepaard met cognitieve problemen. Om deze cognitieve problemen te begrijpen is het belangrijk te weten hoe de normale ontwikkeling van cognitieve vaardigheden verloopt in kinderen. Daarom hebben we in hoofdstuk 5 de relatie tussen een groot aantal verschillende neuropsychologische functies en leeftijd, geslacht en intelligentie onderzocht in een grote steekproef van normaal ontwikkelende kinderen. Zoals verwacht vonden we een sterke relatie met leeftijd, waarbij oudere kinderen beter presteerden dan jongere kinderen op alle cognitieve domeinen. Bij sommige cognitieve functies zagen we dat het functioneren vanaf een bepaalde leeftijd redelijk stabiel bleef, mogelijk erop duidend dat de ontwikkeling van deze functies (bijna) voltooid is. Daarnaast zagen we een duidelijk effect van geslacht, waarbij meisjes beter functioneerden dan jongens, met uitzondering van visuospatiële taken. Tenslotte vonden we dat IQ positief geassocieerd was met neuropsychologisch functioneren, waarbij we de sterkste associatie met visuospatiële taken vonden.

Omdat een efficiënte communicatie tussen verschillende gebieden van de hersenen van groot belang is voor het cognitief functioneren, hebben we in hoofdstuk 6 de relatie tussen witte stof integriteit en neuropsychologisch functioneren onderzocht in een groot populatie-gebaseerde steekproef van jonge kinderen. In dit hoofdstuk laten we zien dat er een positieve relatie bestaat tussen witte stof integriteit en algemeen cognitief functioneren (non-verbaal IQ), als ook met visuospatieel functioneren. Mogelijk wordt onze bevinding van een beter cognitief functioneren gerelateerd aan witte stof integriteit verklaard door meer myelinisatie in het brein en, daardoor, een betere connectiviteit en communicatie tussen hersengebieden.

In hoofdstuk 7 hebben we onderzocht of kinderen met symptomen van onoplettendheid en hyperactiviteit problemen ervaren in het algemeen cognitief functioneren, of dat er sprake is van problemen in meer specifieke cognitieve functies. Daarnaast hebben we onderzocht wat de rol is van de morfologie van de cortex (corticale dikte en gyrificatie), als mogelijke gedeelde neurobiologische substraat onderliggend aan zowel aandachts-/hyperactiviteitsproblemen en cognitieve problemen. In dit hoofdstuk laten we zien dat kinderen met aandachts- en hyperactiviteitsproblemen in de algemene populatie specifieke problemen laten zien op het gebied van executief functioneren in plaats van een algemeen cognitief probleem. Ook vonden we dat meer aandachts- en hyperactiviteitsproblemen en slechter executief functioneren gerelateerd zijn aan een globaal dunnere cortex en minder gyrificatie. Tenslotte laten we zien dat corticale morfologie de relatie tussen aandachts-/hyperactiviteitsproble-

men en problemen in executief functioneren deels verklaart, wat er inderdaad op lijkt te wijzen dat corticale morfologie een gedeelde neurobiologische substraat van deze twee constructen is. Dit suggereert dat executieve functie problemen in ADHD niet als een afzonderlijk cognitief probleem, maar als integraal onderdeel van de stoornis zouden moeten worden beschouwd.

In het laatste deel van dit proefschrift, hoofdstuk 8, wordt een algemene discussie van de belangrijkste bevindingen beschreven. Daarnaast beschrijft dit hoofdstuk een aantal methodologische en praktische implicaties en suggesties voor toekomstig onderzoek.

A

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Publications and manuscripts

PhD portfolio

Dankwoord

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Sabine Elise Mous was born on the 21st of November 1985 in Schiedam, the Netherlands. In 2004 she graduated from secondary school at S.G. Spieringshoek in Schiedam. She went on to study at Leiden University, where she completed her Bachelor of Science Degree in Psychology in 2007. Hereafter, Sabine completed her Master of Science Degree in Clinical Neuropsychology at Leiden University in 2009. Already during her studies, Sabine worked as a research assistant at the department of Child and Adolescent Psychiatry/Psychology within the Generation R Study in the Erasmus Medical Center in Rotterdam. In 2010, she became a PhD student, and started the work described in this thesis. As part of her PhD, Sabine obtained a Master of Science Degree in Clinical Epidemiology from the Netherlands Institute for Health Sciences in 2013. In the last year of her PhD, Sabine worked part-time as a psychologist at the outpatient clinic of the department of Child and Adolescent Psychiatry/Psychology next to her work in scientific research. In January 2015, Sabine started working as a postdoctoral researcher at the department of Child and Adolescent Psychiatry/Psychology within ENCORE (expertisecentrum Erfelijke Neurocognitieve Ontwikkelingsstoornissen, Rotterdam, Erasmus MC), where she studies cognitive and behavioral problems in neurocognitive developmental disorders such as Tuberous Sclerosis Complex, Angelman Syndrome, Fragile X Syndrome, and Neurofibromatosis Type I.

PUBLICATIONS AND MANUSCRIPTS

Mous SE, Hammerschlag AR, Polderman TJC, Verhulst FC, Tiemeier H, van der Lugt A, Jaddoe VW, Hofman A, White T, Posthuma D. A Population-Based Imaging Genetics Study of Inattention/Hyperactivity: Basal Ganglia and Genetic Pathways. *Journal of the American Academy of Child & Adolescent Psychiatry,* accepted for publication.

Mous SE, Schoemaker NK, Blanken LME, Thijssen S, van der Ende J, Polderman TJC, Jaddoe VW, Hofman A, Verhulst FC, Tiemeier H, White T. The Association of Gender, Age and Intelligence with Neuropsychological Functioning in Young Typically Developing Children - The Generation R Study. *Applied Neuropsychology: Child,* accepted for publication.

Muetzel RL, **Mous SE**, van der Ende J, Blanken LME, van der Lugt A, Jaddoe VW, Verhulst FC, Tiemeier H, White T. White matter integrity and cognitive performance in school-age children: The Generation R Study. *Neuroimage*, 2015, epub ahead of print.

Blanken LME, **Mous SE**, Ghassabian A, Muetzel RL, El Marroun H, van der Lugt A, Jaddoe VW, Hofman A, Verhulst FC, Tiemeier H, White T. Cortical thickness and gyrification in 6-to-9 year old children with autistic traits – A population-based imaging study. *American Journal of Psychiatry*, 2015, 172(5), 479-486.

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Serdarevic F, van Batenburg-Eddes T, **Mous SE**, White T, Hofman A, Verhulst FC, Jaddoe VW, Ghassabian A, Tiemeier H. Relation of Infant Motor Development with Nonverbal Intelligence, Language Comprehension and Neuropsychological Functioning in Childhood. A population-based study. *Developmental Science*, accepted for publication.

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Supervisor(s):	Dr. T. White

1. PHD TRAINING

GENERAL COURSES

Masters degree Health Sciences, specialisation Clinical Epidemiology, NIHES,
Erasmus University Rotterdam, the Netherlands

	YEAR	WORKLOAD (ECTS)
Erasmus Summer Programme:		
Principles of Research in Medicine and Epidemiology	2011	0.7
Clinical Decision Analysis	2012	0.7
Methods of Public Health Research	2012	0.7
Topics in Meta-analysis	2012	0.7
Pharmaco-epidemiology	2012	0.7
Genome Wide Association Analysis	2011	1.4
Principles of Genetic Epidemiology	2011	0.7
Genomics in Molecular Medicine	2011	1.4
Markers and Prognostic Research	2012	0.7
Advances in Genomics Research	2011	0.4
The Practice of Epidemiologic Analysis	2012	0.7
Core Curriculum:		
Study Design	2011	4.3
Classical Methods for Data-analysis	2012	5.7
Clinical Epidemiology	2011	5.7
Methodologic Topics in Epidemiologic Research	2011	1.4
Biostatistical Methods II: Classical Regression Models	2012	4.3
Advanced courses:		
Repeated Measurements in Clinical Studies	2012	1.4
Missing Values in Clinical Research	2012	0.7
Courses for the Quantitative Researcher	2012	1.4
Principles of Epidemiologic Data-analysis	2012	0.7

	YEAR	WORKLOAD (ECTS)
SPECIFIC COURSES		
MRI Safety course, Erasmus MC, Rotterdam, the Netherlands	2010	0.3
Endnote course, Erasmus MC, Rotterdam, the Netherlands	2010	0.3
Basiscursus Regelgeving en Organisatie Klinische trials, Erasmus MC, Rotterdam, the Netherlands	2011	1.0
FreeSurfer course, Amsterdam, the Netherlands	2012	1.0
Introduction to Imaging Genetics, OHBM, Seattle, USA	2013	0.3
Training 'Oriëntatie op je Loopbaan', Erasmus MC, Rotterdam, the Netherlands	2014	0.7
FSL & FreeSurfer course, Oxford, UK	2014	2.0
INTERNATIONAL CONFERENCES		
Human Brain Mapping, Seattle, USA (poster presentation)	2013	1.2
Human Brain Mapping, Hamburg, Germany (poster presentation)	2014	1.2
Society for Research in Child Development, Philadelphia, USA (poster presentation)	2015	0.9
WORKSHOPS, MEETINGS AND SYMPOSIA		
Symposium 'Neuroimaging, Genetics and Endophenotypes: Development and Psychopathology', Rotterdam, the Netherlands	2010	0.3
Symposium 'Brain Development and Developmental Disorders', Utrecht, the Netherlands	2012	0.3
Neuroscience Campus Amsterdam Annual Meeting, Amsterdam, the Netherlands (poster presentation)	2013	0.3
Symposium 'Sophia 150 years: children of the future', Rotterdam, the Netherlands	2013	0.3
VUMC Science Exchange Day, Amsterdam, the Netherlands (poster presentation)	2014	0.3
Sophia Research Days, Rotterdam, the Netherlands (oral and poster presentation)	2013-2014	0.6
NWO symposia 'Brain and Cognition', Utrecht, the Netherlands (poster and oral presentations, 1 award winning)	2010-2014	1.2
BIGR-KNICR MRI meetings, Erasmus MC, Rotterdam, the Netherlands (1 oral presentation)	2010-2014	1.0
CTG Lab journal club, VU University, Amsterdam, the Netherlands (2 oral presentations)	2010-2014	1.0
Generation R research meetings, Erasmus MC, Rotterdam, the Netherlands (1 oral presentation)	2010-2014	1.0

2. TEACHING ACTIVITIES

SUPERVISING MASTER'S THESES		
Nikita Schoemaker (Clinical Neuropsychology, Leiden University)	2011	3.0
The influence of maternal folate blood level during pregnancy on memory in 6-7 year old children		
Karin Immink (Child- and Adolescent Psychology, Leiden University)	2012	3.0
The influence of maternal vitamin B12 level during pregnancy on memory in children		
Loes Asmus (Clinical Neuropsychology, Maastricht University)	2012	3.0
Volumetric differences in the limbic system are visible in children with Severe Mood Dysregulation: an imaging study		
Lesley Woldendorp (Clinical Neuropsychology, Leiden University)	2013	3.0
The relationship between corpus callosum size and attention problems		
Toesja Klein (Clinical Neuropsychology, Leiden University)	2013	3.0
The relationship between reported ADHD symptoms and neurocognitive performance in the areas of attention and executive functioning in young children		
Sanderein Snel (Clinical Neuropsychology, Leiden University)	2014	3.0
Verbal fluency and brain structures related to language and executive functioning in young children with Attention Deficit Hyperactivity Disorder		

	YEAR	WORKLOAD (ECTS)
OTHER TEACHING ACTIVITIES		
Lecture pediatric neuroimaging research, Adolescent clinic, Erasmus MC, Rotterdam, the Netherlands	2010	0.3
Lecture pediatric neuroimaging research, CED group, Rotterdam, the Netherlands	2012	0.3
Supervising and lecturing 2nd year medical students, Erasmus University, Rotterdam, the Netherlands	2013	1.0
Supervising workgroup 3rd year medical students, Erasmus University, Rotterdam, the Netherlands	2014	0.3
Het Familiealbum (Wetenschapsknooppunt EUR), teaching elementary school children about scientific research, Rotterdam, the Netherlands	2012-2013	1.0
Supervising and lecturing 3rd year EUR medical students, Erasmus University, Rotterdam, the Netherlands	2013-2014	1.0

3. OTHER ACTIVITIES

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Organization of symposium 'Neuroimaging, Genetics and Endophenotypes: Development and Psychopathology'	2010	1.0
Part of NWO Brain and Cognition workgroup Neuroimaging Datasharing	2014	1.2
Reviewing article Psychiatry Research Neuroimaging	2014	0.2

1 ECTS (European Credit Transfer System) is equal to a workload of 28 hours.

DANKWOORD

Na ruim vier jaar aan mijn promotieonderzoek te hebben gewerkt, voelt het schrijven van dit dankwoord bijzonder en tegelijk ook een beetje onwerkelijk. Het zit erop, het werk is gedaan en het proefschrift is klaar! Werk dat ik nooit helemaal alleen had kunnen doen. Daarom is dit laatste deel van mijn proefschrift bestemd voor alle mensen om mij heen die mij hierbij hebben geholpen en die ik daarvoor heel dankbaar ben!

Om te beginnen wil ik alle kinderen en ouders die al die jaren meedoen aan het Generation R onderzoek heel hartelijk bedanken. Zonder de vele bezoekjes die jullie hebben afgelegd aan ons centrum en de grote aantallen vragenlijsten die jullie altijd zo trouw hebben ingevuld, was dit onderzoek niet mogelijk geweest.

Graag wil ik ook mijn promotoren Prof.dr. Henning Tiemeier, Prof.dr. Frank Verhulst, Prof.dr. Danielle Posthuma en co-promotor Dr. Tonya White bedanken voor de begeleiding. Beste Henning, bedankt voor jouw altijd kritische commentaar op stukken. Ik heb in de afgelopen jaren erg veel van jou geleerd over het doen van goed epidemiologisch onderzoek. Ik kan mij nog goed herinneren dat ik voor het eerst een gedragsgroep meeting bijwoonde en onder de indruk (en ook wel een beetje geïntimideerd) was door jouw grote epidemiologische kennis en enthousiasme voor het onderzoek. Beste Frank, hartelijk bedankt voor het meedenken over niet alleen mijn manuscripten, maar ook mijn verdere carrière. Het was altijd erg fijn hoe vlot jij op stukken reageerde en deze van waardevolle opmerkingen vanuit een klinisch perspectief voorzag. Ik heb het daarnaast als erg prettig ervaren dat je de tijd nam om met mij te praten over mijn verdere loopbaan en de keuze tussen wetenschappelijk onderzoek en klinisch werk. Beste Danielle, de combinatie van neuropsychologie en neuroimaging is wat mij betreft niet compleet zonder de genetica erbij te betrekken. Heel erg bedankt dat je mij, als relatieve leek op het gebied van genetica, tijdens mijn promotietraject deze kennis hebt bijgebracht en mij een kijkje hebt gegeven in dit interessante onderzoeksgebied! Beste Tonya, zonder jou was de Generation R 'pilot' neuroimaging studie, en dus ook dit proefschrift, er nooit geweest. Ik heb het een eer gevonden om vrijwel vanaf de start, vlak na jouw verhuizing naar Nederland, deel te zijn geweest van jouw KNICR groep en het neuroimaging onderzoek mede op te hebben mogen zetten binnen Generation R. Hartelijk bedankt voor je hulp en het delen van je grote neuroimaging kennis!

Graag wil ik Prof.dr. Ype Elgersma, Prof.dr. Aad van der Lugt en Prof.dr. Sarah Durston hartelijk bedanken voor de bereidheid zitting te nemen in de kleine commissie en het beoordelen van mijn proefschrift. Dr. Marjolein Wals en Dr. Liesbeth Reneman, hartelijk bedankt voor het plaatsnemen in de grote commissie en de aanwezigheid tijdens mijn verdediging. Dr. Steven Chance, thank you very much for your willingness to participate in my dissertation committee.

Ook wil ik graag al mijn co-auteurs bedanken voor de fijne samenwerking! Bedankt voor jullie goede ideeën, kritische commentaren en hulp!

Dan een woord van dank aan mijn paranimfen. Lieve Laura, dank je wel dat je mijn paranimf wilde zijn! Ik heb in de afgelopen jaren genoten van onze samenwerking. Je bent een harde werker, kritische denker, maar bovenal ook een hele fijne en gezellige kamergenoot. Ondanks dat we nu geen kamergenoten meer zijn hoop ik je nog vaak te zien en spreken! Binnenkort weer een keer whisky proeven? Lieve Daphne, natuurlijk zou jij een van mijn paranimfen zijn! Je bent niet alleen mijn zusje, maar ook mijn beste vriendin en zelfs ook nog eens een collega in de wetenschap en in het Erasmus MC! Ik heb respect voor hoe hard jij werkt en hoop ook snel getuige te mogen zijn van jouw verdediging.

Dat achter de schermen bij een grootschalig onderzoek als Generation R heel veel gebeurt en geregeld wordt, hoef ik denk ik niet uit te leggen. Prof.dr. Vincent Jaddoe, Prof.dr. Albert Hofman en de overige leden van het management team; hartelijk bedankt voor het opzetten van dit schitterende onderzoek! Patricia en Rose, bedankt voor jullie onmisbare secretariële ondersteuning. Claudia en Marjolein, heel erg bedankt voor de altijd vlotte aanlevering van datasets. Alwin, bedankt voor je hulp bij computerproblemen. Karien, Ronald en Natalia, ook jullie heel erg bedankt voor de fijne samenwerking! Ook wil ik Erica bedanken; het is niet altijd makkelijk om een afspraak te plannen in de agenda van Henning, maar het lukt je toch altijd weer. En natuurlijk ook een woord van dank aan Laureen; zonder al jouw kennis en onmisbare hulp had het regelen van alle zaken rond mijn promotie een heel stuk minder makkelijk geweest. Je bent een topper! Lieve Focusdames, ook jullie wil ik hartelijk bedanken voor de samenwerking. In het bijzonder wil ik de dames van het gedragsblok noemen; Anneke, Ineke, Rukiye, Sabah en Tonie, bedankt voor de gezelligheid tijdens de vele uren die we samen op het Focuscentrum hebben doorgebracht en jullie grote inzet voor het onderzoek!

Onderzoek doe je niet alleen, maar is een echte teamsport! Graag wil ik in dit verband mijn collega's van de KNICR neuroimaging groep noemen. Lieve Akvile, Alette, Andrea, Charlotte, Gerbrich, Hanan, Ilse, Laura, Marcus, Nikita, Ryan, Sandra (Langeslag) en Sandra (Thijssen); het heeft heel wat bloed, zweet, tranen en weekenden gekost, maar we hebben het met elkaar toch voor elkaar gekregen om ruim 1.000 kinderen te scannen en uitgebreid neuropsychologisch onderzoek te doen! Lieve Akhgar, Carolyn, Desana, Maja, Monica, Philip en Raisa; ook jullie hartelijk bedankt voor de fijne samenwerking! Ook kijk ik met veel plezier terug op de buitenlandse bezoeken die ik met een deel van de groep heb mogen meemaken; OHBM in Hamburg en de FreeSurfer cursus in Oxford waren beide (buiten erg leerzaam uiteraard…) ook heel erg gezellig! Nooit geweten dat je op deze leeftijd nog steeds zoveel plezier kunt hebben in een speeltuin! Natuurlijk wil ik ook alle studenten die hun steentje hebben bijgedragen aan onze dataverzameling heel hartelijk bedanken! In het bijzonder wil ik daarbij onze studenten van het eerste uur Anouk, Kary, Madhvi en Elles noemen.

Aan alle collega's op de afdelingen Generation R en de Kinder- en Jeugdpsychiatrie/psychologie in het Erasmus MC en het CTG lab aan de VU; bedankt voor de samenwerking, support, gezamenlijke lunches, borrels en koffiemomentjes! In het bijzonder wil ik mijn kamergenoten bedanken. Lieve Jolien, het was super om met jou kamer Ae-005 te hebben mogen delen! Als newbie op de afdeling was het heel fijn om bij iemand op de kamer te komen die zo vriendelijk en behulpzaam is als jij. Bedankt daarvoor, net als voor alle gezellige momenten die we samen (zingend) op de kamer doorbrachten! Dear Ryan, you're simply the best! I truly don't know any other person so helpful and friendly as you are! Our group, and Generation R, is very lucky to have you. Thanks for being such a great friend! Lieve Alette, ook al was het maar kort, ook jij bedankt voor de tijd samen in Ae-005! Het was leuk om, na de adolescenten kliniek, weer met je te mogen samenwerken! Lieve kamergenootjes van NA-2809; Dafna, Ana, Iolanda, Michelle en Philip, heel erg bedankt voor de fijne sfeer. In het bijzonder mijn 'eilandgenoten'; lieve Andrea, jij bent altijd vrolijk en positief, heel fijn zo'n kamergenoot! Lieve Laura, bedankt voor de goede discussies en overlegmomentjes! Aan mijn collega's van de polikliniek Kinder- en Jeugdpsychiatrie; bedankt dat ik de kans heb gekregen om in mijn laatste jaar de wetenschap en kliniek met elkaar af te wisselen. Ik ben blij dat ik nu, in mijn nieuwe functie, weer deel mag uitmaken van jullie gezellige team!

Ook veel dank aan mijn lieve vrienden en vriendinnen voor de broodnodige ontspanning en support! In het bijzonder wil ik Daphne, Arno, Wilianne en Marc noemen. Bedankt voor jullie support en interesse en natuurlijk voor de altijd gezellige en bovenal lekkere 'kookclub' etentjes! Lieve Sara-Mae, Sander, Alessandra, Donovan, Masha en Fedor; ook jullie bedankt voor de gezellige avonden samen! Echt meiden, ooit gaat het ons lukken om '30 seconds' te winnen van de mannen! Lieve Marije, dankzij jou ben ik ooit gaan nadenken over het doen van promotieonderzoek, bedankt voor dat duwtje in de goede richting! Ik hoop dat we elkaar nog lang zullen blijven zien om over onze wetenschappelijke en niet-wetenschappelijke leven te kletsen! Lieve Pitchers, ook jullie heel erg bedankt. Zingen is mijn grootste hobby en het is fijn om onderdeel te zijn van zo'n liefdevolle muzikale familie waarin je samen kunt doen waar je het meeste van geniet!

Tenslotte heel veel dank aan mijn familie en in het bijzonder aan mijn ouders. Lieve pap en mam, bedankt dat jullie er altijd voor mij en Daphne zijn! Bedankt voor alle kansen die jullie ons gegeven hebben en jullie eeuwige steun en interesse voor wat wij doen. Lieve Joke en Joop, je schoonouders krijg je er gratis bij, maar als ik had kunnen kiezen dan had ik het zeker niet anders gedaan. Bedankt voor jullie gezelligheid en positiviteit! Lieve Daf en Paul, lieve Martin en Fabienne; bedankt voor jullie support en alle gezellige momenten samen! Lieve oma's, wat ben ik blij dat jullie dit voor mij heel belangrijke moment hebben mogen meemaken. Bedankt voor jullie steun! En dan de allerlaatste persoon uit dit dankwoord. Liefste Frank, zonder jou was ik nooit zover gekomen. Je bent mijn motivator, luisterend oor en steun en toeverlaat! Jouw rust, relativeringsvermogen en liefde zijn onmisbaar geweest. Bedankt dat je er altijd voor me bent! Ik hou van jou!

ACKNOWLEDGEMENTS

The Generation R Study is conducted by the Erasmus Medical Centre Rotterdam in close collaboration with the Faculty of Social Sciences of the Erasmus University Rotterdam, the Municipal Health Service Rotterdam, and the Stichting Trombosedienst & Artsenlaboratorium Rijnmond (STAR), Rotterdam. We gratefully acknowledge the contribution of all participating children and their families, general practitioners, hospitals, midwives, and pharmacies in Rotterdam. The general design of the Generation R Study is made possible by the Erasmus Medical Centre Rotterdam, the Erasmus University Rotterdam, the Netherlands Organisation for Health Research and Development (ZonMw), the Netherlands Organisation for Scientific Research (NWO), the Ministry of Health, Welfare and Sport, and the Ministry of Youth and Families.

The work presented in this thesis was conducted at the Department of Child and Adolescent Psychiatry/Psychology of the Erasmus Medical Centre – Sophia's Children Hospital in Rotterdam, the Netherlands. Studies were financially supported by grants from NWO (Brain & Cognition, 433-09-228), NWO/ZonMw (TOP, 91211021), NWO (VICI, 453-14-005), the European Community's 7th Framework Programme (FP7/2008-2013, 212652), the Sophia Children's Hospital Research Foundation (SSWO) (project 639), and the Brain and Behavior Research Foundation via the Blowitz-Ridgeway Foundation (NIMH K08 MH068540). Supercomputing resources were supported by the NWO Physical Sciences Division (Exacte Wetenschappen) and SURFsara (Lisa compute cluster, www.surfsara.nl). Financial support for the publication of this thesis was provided by the Department of Child and Adolescent Psychiatry/Psychology, the Generation R Study, and the Erasmus Medical Centre.

The Distracted Brain

This thesis focuses on the neurobiology and neuropsychology of attention-deficit/hyperactivity problems in the general population.

The notion that child psychopathology might be better described within a dimensional framework, rather than with clearly defined diagnostic categories, has recently gained support. Despite this, the majority of studies are still performed in clinical samples of children with attention-deficit/hyperactivity disorder (ADHD). By studying the neurobiology (neuroimaging and genetics) and neuropsychology of attention-deficit/hyperactivity problems in the general population, this thesis extends previous work in clinical samples to the full range of problems in the general population.